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(54) Title: PARATHYROID HORMONE RECEPTOR AND DNA ENCODING SAME

## (57) Abstract

DNA encoding a parathyroid hormone receptor; production and isolation of recombinant and synthetic parathyroid hormone receptor polypeptides and fragments; antibodies to parathyroid hormone receptors and receptor fragments; methods for screening candidate compounds for antagonistic or agonistic effects on parathyroid hormone receptor action; and diagnostic and therapeutic methods of these compounds are disclosed.

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**PARATHYROID HORMONE RECEPTOR AND DNA ENCODING SAME**

Background of the Invention

Partial funding of the work described herein was  
5 provided by the U.S. Government, which has certain rights  
to the invention.

The invention relates to endocrine receptors.

A crucial step in the expression of hormonal action is the interaction of hormones with receptors on  
10 the plasma membrane surface of target cells. The formation of hormone-receptor complexes allows the transduction of extracellular signals into the cell to elicit a variety of biological responses. For example, binding of a hormone such as follicle stimulating hormone  
15 (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), and chorionic gonadotropin (CG), to its cell surface receptor induces a conformational change in the receptor, resulting in the association of the receptor with a transductor molecule, the stimulatory  
20 guanine nucleotide (GTP) binding protein, a component of which is ( $G_s$ ). This association stimulates adenylate cyclase activity which in turn triggers other cellular processes such as protein phosphorylation, steroid synthesis and secretion, and the modulation of ion flux.  
25 Binding of other hormones, including arginine vasopressin (VP), angiotensin II, and norepinephrine, to their cell surface receptors results in the activation of other types of GTP binding proteins components such as ( $G_p$ ), which in turn stimulates the activity of the enzyme  
30 phospholipase C. The products of phospholipase C hydrolysis initiate a complex cascade of cellular events, including the mobilization of intracellular calcium and protein phosphorylation.

Parathyroid hormone (PTH) is a major regulator of calcium homeostasis whose principal target cells occur in

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bone and kidney. Regulation of calcium concentration is necessary for the normal function of the gastrointestinal, skeletal, neurologic, neuromuscular, and cardiovascular systems. PTH synthesis and release

5 are controlled principally by the serum calcium level: a low level stimulates and a high level suppresses both the hormone synthesis and release. PTH, in turn, maintains the serum calcium level by directly or indirectly promoting calcium entry into the blood at three sites of

10 calcium exchange: gut, bone and kidney. PTH contributes to net gastrointestinal absorption of calcium by favoring the renal synthesis of the active form of vitamin D. PTH promotes calcium resorption from bone by inhibiting osteoblasts and, indirectly, by stimulating

15 differentiation of the bone-resorbing cells, osteoclasts. It also mediates at least three main effects on the kidney: stimulation of tubular calcium reabsorption, enhancement of phosphate clearance, and promotion of an increase in the enzyme that completes synthesis of the

20 active form of vitamin D. PTH exerts these effects primarily through receptor-mediated activation of adenylate cyclase, although receptor-mediated activation of phospholipase C by PTH has also been reported (Hruska et al., J. Clin. Invest. 79:230, 1987).

25 Disruption of calcium homeostasis may produce many clinical disorders (e.g., severe bone disease, anemia, renal impairment, ulcers, myopathy, and neuropathy) and usually results from conditions which produce an alteration in the level of parathyroid hormone.

30 Hypercalcemia is a condition which is characterized by an elevation in the serum calcium level. It is often associated with primary hyperparathyroidism in which an excess of PTH production occurs as a result of a lesion (e.g., adenoma, hyperplasia or carcinoma) of the

35 parathyroid glands. Another type of hypercalcemia,

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humoral hypercalcemia of malignancy (HHM), is the most common paraneoplastic syndrome. It appears to result in most instances from the production by tumors (e.g., squamous, renal, ovarian or bladder carcinomas) of a 5 novel class of protein hormone which shares amino acid homology with PTH. These PTH-related proteins (PTHrP) appear to mimic certain of the renal and skeletal actions of PTH and are believed to interact with the PTH receptor in these tissues. PTHrP is normally found at low levels 10 in many tissues, including keratinocytes, brain, pituitary, parathyroid, adrenal cortex, medulla, fetal liver, osteoblast-like cells and lactating mammary tissues. In many HHM malignancies, PTHrP is found in the circulatory system at high levels, thereby producing the 15 elevated calcium levels associated with HHM.

Summary of the Invention

The invention features isolated DNA comprising a DNA sequence encoding a cell receptor, preferably a parathyroid hormone receptor, of a vertebrate animal, 20 which receptor has an amino acid sequence with at least 30% (preferably at least 50%, even more preferably at least 60%, and most preferably at least 75%) identity to the amino acid sequence shown in FIG. 3 (SEQ ID NO.: 3): i.e., when the closest match is made between the two 25 amino acid sequences (using standard methods), at least 30% of the amino acid residues of the former sequence are identical to the amino acid residues of the latter sequence. By "isolated" is meant that the DNA is free of the coding sequences of those genes that, in the 30 naturally-occurring genome of the organism (if any) from which the DNA of the invention is derived, immediately flank the gene encoding the DNA of the invention. The isolated DNA may be single-stranded or double-stranded, and may be genomic DNA, cDNA, recombinant hybrid DNA, or

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synthetic DNA. It may be identical to a naturally-  
occurring, cell receptor- (e.g. PTH receptor) encoding  
DNA sequence, or may differ from such sequence by the  
deletion, addition, or substitution of one or more  
5 nucleotides. Single-stranded DNAs of the invention are  
generally at least 8 nucleotides long, (preferably at  
least 18 nucleotides long, and more preferably at least  
30 nucleotides long) ranging up to full length of the  
gene or cDNA; they preferably are detectably labelled for  
10 use as hybridization probes, and may be antisense.  
Preferably, the isolated DNA hybridizes under conditions  
of high stringency to all or part of the DNA sequence  
shown in FIG. 1 (SEQ ID NO.:1), FIG. 2 (SEQ ID NO.:2),  
FIG. 3 (SEQ ID NO.:3), or FIG. 6 (SEQ ID NO.:4). By  
15 "high stringency" is meant, for example, conditions such  
as those described herein below for the isolation of  
human kidney PTH receptor cDNA (also see Current  
Protocols in Molecular Biology, John Wiley & Sons, New  
York, 1989, hereby incorporated by reference). Most  
20 preferably, the animal is a mammal (such as an opossum, a  
rat, or a human), and the DNA sequence encodes  
substantially all of the amino acid sequence shown in  
FIG. 1 (SEQ ID NO.:1), FIG. 2 (SEQ ID NO.:2), FIG. 3 (SEQ  
ID NO.:3) or FIG. 6 (SEQ ID NO.:4); or is encoded by the  
25 coding sequence of one of the plasmids deposited with the  
American Type Culture Collection (ATCC) and designated  
ATCC Accession No. 68570 or 68571. The DNA of the  
invention may be incorporated into a vector [which may be  
provided as a purified preparation (e.g., a vector  
30 separated from the mixture of vectors which make up a  
library)] containing a DNA sequence encoding a cell  
receptor of the invention (e.g. parathyroid hormone  
receptor) or fragment of the receptor, and a cell or  
essentially homogenous population of cells (e.g.,  
35 prokaryotic cells, or eukaryotic cells such as mammalian

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cells) which contain the vector (or the isolated DNA described above). By "essentially homogenous" is meant that at least 99% of the cells contain the vector of the invention (or the isolated DNA, as the case may be).

5 Preferably, this vector (e.g., R15B) is capable of directing expression of a parathyroid hormone receptor (for example, in a cell transfected or transformed with the vector).

In another aspect, the invention features a cell  
10 receptor, preferably parathyroid hormone receptor, (or an essentially purified preparation thereof) produced by expression of a recombinant DNA molecule encoding the cell receptor. An "essentially purified preparation" is one which is substantially free of the proteins and  
15 lipids with which it is naturally associated.

In a related aspect, the invention features a polypeptide which includes a fragment of a naturally-occurring cell receptor of the invention. Preferably, the polypeptide includes a fragment of a naturally-  
20 occurring parathyroid hormone receptor which is capable of binding parathyroid hormone or parathyroid hormone-related protein. In preferred embodiments, this fragment is at least six amino acids long, and has a sequence selected from the group including:

25 (a) TNETREREVFDRLGMIYTVG; (SEQ ID NO.: 5)  
(b) YLYSGFTLDEAERLTEEEL; (SEQ ID NO.: 6)  
(c) VTFFFLYFLATNYYWILVEG; (SEQ ID NO.: 7)  
(d) Y-RATLANTGCWDLSSGHKKWIIQVP; (SEQ. ID NO.: 8)  
(e) PYTEYSGTLWQIQMHYEM; (SEQ ID NO.: 9)  
30 (f) DDVFTKEEQIFLLHRAQA; (SEQ ID NO.: 10)  
(g) FFRLHCTRNY; (SEQ ID NO.: 11)  
(h) EKKYLWGFTL; (SEQ ID NO.: 12)  
(i) VLATKLRETNAGRCDTRQQYRKLLK; or (SEQ ID NO. 13)  
(j) a fragment (i.e., a portion at least six  
35 residues long, but less than all) or analog of (a) - (i)

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which is capable of binding parathyroid hormone or parathyroid hormone-related protein [wherein "analog" denotes a peptide having a sequence at least 50% (and preferably at least 70%) identical to the peptide of 5 which it is an analog]. Preferably, the polypeptide of the invention is produced by expression of a recombinant DNA molecule or is synthetic (i.e., assembled by chemical rather than biological means). The invention provides a method for producing such a polypeptide, which method 10 includes providing a cell containing isolated DNA encoding a cell receptor of the invention or receptor fragment and culturing this cell under conditions which permit expression of a polypeptide from the isolated DNA.

The invention also features an antibody 15 (monoclonal or polyclonal), and a purified preparation of an antibody, which is capable of forming an immune complex with a cell receptor of the invention (preferably a parathyroid hormone receptor such as a human PTH receptor) such antibody being generated by using as 20 antigen either (1) a polypeptide that includes a fragment of the cell receptor of the invention, or (2) a cell receptor of the invention which is on the surface of a cell. This antibody is preferably capable of neutralizing (i.e., partially or completely inhibiting) a 25 biological activity of the cell receptor of the invention (i.e., a component of one of the cascades naturally triggered by the receptor when its ligand binds to it). In preferred embodiments, the antibody of the invention is capable of forming an immune complex with parathyroid 30 hormone receptor and is capable of neutralizing a biological activity of the PTH receptor (i.e. adenylate cyclase activation or phospholipase C stimulation)

Also within the invention is a therapeutic composition including, in a pharmaceutically-acceptable 35 carrier, (a) a cell receptor of the invention, (b) a

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polypeptide containing a fragment of the cell receptor of the invention, or (c) an antibody to a cell receptor of the invention. These therapeutic compositions provide a means for treating various disorders characterized by  
5 overstimulation of the cell receptors of the invention by their ligand. In preferred embodiments, the polypeptides of the invention include the PTH receptor, fragments of the PTH receptor and antibodies which form immune complexes with the PTH receptor. These polypeptides and  
10 antibodies are useful as diagnostics, for distinguishing those cases of hypercalcemia related to PTH or PTHrP from those which are not.

The nucleic acid probes of the invention enable one of ordinary skill in the art of genetic engineering  
15 to identify and clone cell receptor homologs or cell receptors from any species which are related to the cell receptors of the invention, expanding the usefulness of the sequences of the invention.

Other features and advantages of the invention  
20 will be apparent from the following description of the preferred embodiments and from the claims.

#### Detailed Description

The drawings will first be briefly described.

#### DRAWINGS

25 FIG. 1 is a representation of the nucleic acid and amino acid sequence encoding the opossum kidney PTH/PTHrP receptor clone, OK-H. (SEQ ID NO.: 1)

FIG. 2 is a representation of the nucleic acid and amino acid sequence encoding the opossum kidney PTH/PTHrP  
30 receptor clone, OK-O. (SEQ ID NO.: 2)

FIG. 3 is a representation of the nucleic acid and amino acid sequence encoding the rat bone PTH/PTHrP receptor clone, R15B. (SEQ ID NO.: 3)

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FIG. 4 is a comparison of the deduced amino acid sequences encoded by cDNAs from clones OK-O and R15B.

FIG. 5 is a comparison of the deduced amino acid sequences of OK-O, OK-H and R15B, lined up according to 5 sequence homology.

FIG. 6 is a representation of the nucleic acid and amino acid sequence encoding the human PTH/PTHrP receptor. (SEQ ID NO.: 4)

FIG. 7 is a schematic representation of the rat 10 bone PTH/PTHrP receptor cDNA, the human genomic DNA clone HPG1 and two cDNA clones encoding the human PTH/PTHrP receptor.

FIG. 8 is a hydrophobicity plot of the deduced 15 amino acid sequence of the human kidney PTH/PTHrP receptor. Predicted membrane-spanning domains I through VII are indicated; A, B and C indicate additional hydrophobic regions.

FIG. 9 is a graph illustrating binding of PTHrP to COS cells transfected with OK-H.

20 FIG. 10 is a graph illustrating stimulation of intracellular free calcium by NlePTH in COS cells transfected with OK-H.

FIG. 11 is a graph illustrating binding of PTHrP to COS cells transfected with OK-O.

25 FIG. 12 is a graph illustrating stimulation of intracellular free calcium by NlePTH in COS cells transfected with OK-O.

FIG. 13 is a graph illustrating binding of PTHrP to COS cells transfected with R15B.

30 FIG. 14 is a graph illustrating stimulation of intracellular free calcium by NlePTH in COS cells transfected with R15B.

FIG. 15 is a graph illustrating stimulation of inositol phosphate metabolism by NlePTH in COS cells 35 transfected with OK-H, OK-O, or R15B.

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FIG. 16 is a graph illustrating cyclic AMP accumulation in COS cells transfected with CDM-8, OK-H, R15B by NlePTH.

FIG. 17 are graphs illustrating binding of  $^{125}\text{I}$ -labelled PTH(1-34) (A and B) and  $^{125}\text{I}$ -labelled PTHrP(1-36) (C and D) to COS-7 cells transiently expressing the human kidney (A and C) and the rat bone (B and D) PTH/PTHrP receptor; competing ligands included PTH(1-34) ( $\square$ ), PTHrP(1-36) (\*), PTH(3-34) (■), PTH(7-34) (+). Data are given as % specific binding and represent the mean $\pm$ SD of at least three independent experiments.

FIG. 18 is a bar graph illustrating stimulated accumulation of intracellular cAMP in COS-7 cells transiently expressing the human kidney receptor. Data show the mean $\pm$ SD, and are representative of at least three independent experiments.

FIG. 19 represents a Northern blot analysis of total RNA (~ 10  $\mu\text{g}/\text{lane}$ ) prepared from human kidney (A) and SaOS-2 cells (B). The blot was hybridized with the full length cDNA encoding the human kidney PTH/PTHrP receptor; positions of 28S and 18S ribosomal RNA bands are indicated.

FIG. 20 represents a Southern blot analysis of human genomic DNA digested with SstI, HindIII, and XbaI (~ 10  $\mu\text{g}/\text{lane}$ ). The blot was hybridized with the full length cDNA encoding the human kidney PTH/PTHrP receptor.

FIG. 21 is a schematic diagram of the proposed arrangement, in a cellular membrane, of PTH/PTHrP rat bone receptor encoded by R15B.

30

#### MATERIALS AND METHODS

GENERAL: [Nle<sup>8,18</sup>, Tyr<sup>34</sup>]bPTH(1-34)amide (PTH(1-34)), [Nle<sup>8,18</sup>, Tyr<sup>34</sup>]bPTH(3-34)amide (PTH(3-34)), and [Nle<sup>8,18</sup>, Tyr<sup>34</sup>]bPTH(7-34)amide (PTH(7-34)) were obtained from Bachem Fine Chemicals, Torrance, CA; [Tyr<sup>36</sup>]PTHrP(1-

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36) amide (PTHrP(1-36)) was synthesized as described (Keutman et al., Endocrinology 117:1230, 1985) using an Applied Biosystems Synthesizer 420A. Dulbecco's modified Eagles medium (DMEM), EDTA/trypsin, and gentamycin were  
5 from GIBCO (Grand Island, NY); fetal bovine serum (FBS) was from Hiclone Laboratory, Logan, UT. Total RNA from human kidney was provided by Per Hellman, University Hospital, Uppsala, Sweden. Oligonucleotide primers were synthesized using an Applied Biosystems 380B DNA  
10 Synthesizer. Restriction enzymes, Klenow enzyme, T4 polynucleotide Kinase and T4 DNA ligase were from New England Biolabs, Beverly, MA. Calf alkaline phosphatase was from Boehringer Mannheim, Germany. All other reagents were of highest purity available.

15 CELLS

Cell lines used include COS cells, OK cells, SaOS-2 cells, CHO cells, AtT20 cells, LLC-PK1 cells, and UMR-106 cells, which are available from a variety of sources including the American Type Culture Collection (Rockland, Maryland), Accession Nos. CRL1650, CRL6551, HTB85, CCL61, CCL89, CL101, and CRL1161, respectively. ROS 17/2 and ROS 17/2.8 are available from a number of sources including Dr. Gideon Rodan (Merck Laboratories, West Point, PA). MC-3T3 cells are derived from mouse bone  
25 cells and are also available from a number of sources including Dr. Chohei Shigeno (Dept. of Biochem. Medicine, Hyoto Univ., Kyoto, Japan).

All cells were grown in a humidified 95% air, 5% CO<sub>2</sub> atmosphere and maintained in monolayer culture with  
30 Ham's F-12 or DMEM medium (Grand Island Biological Co.), supplemented with 5% or 10% fetal calf serum (M.A. Bioproducts, Walkersville, MD). The medium was changed every 3 or 4 days, and the cells were subcultured every 2  
35 or 3 weeks by

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trypsinization using standard methods.

CLONING

Isolation of cDNA clones encoding the rat and opossum PTH/PTHrP receptors: Total RNA was initially isolated from rat osteosarcoma (ROS) cells (ROS 17/2.8) and opossum kidney (OK) cells, by standard methods using guanidium isothiocyanate (Ullrich et al., *Science* 196: 1313, 1977; Chirgwin et al. *Biochemistry* 24: 5294, 1979), and centrifugation through cesium chloride (Gilsen et al., *Biochemistry* 13: 2633, 1974). Poly A+ RNAs (mRNAs) were then recovered after passage of the total RNAs over oligo dT columns (Pharmacia, Piscataway, NJ) by the method of Aviv and Leder (*Proc. Natl. Acad. Sci. USA* 69: 14087, 1972). The cDNA library from the ROS 17/2.8 mRNA was prepared from poly A+ RNA using the method of Gubler and Hoffman (*Gene (Amst.)* 25: 263, 1983). Oligo dT-primed and random-primed cDNAs were synthesized from poly A+ ROS 17/2.8 and OK cell mRNA, respectively (Aviv and Leder, *supra*). The cDNAs were ligated to *BstX*1 linkers (Invitrogen, San Diego, CA) and size-selected by centrifugation (3 h, 55,000 xg) in a 5-20% potassium acetate gradient. The size-selected cDNA was then inserted into the plasmid vector, pcDNA I (Invitrogen), using the non-self annealing *BstX*1 restriction sites. The resultant plasmid libraries were then used to transform *E. coli* (MC1061/P3, Invitrogen) containing a larger helper plasmid, p3. The p3 plasmid possesses amber mutations in two genes which code for ampicillin and tetracycline resistance. Using ampicillin and tetracycline selection, only those cells containing both the p3 and a tRNA suppressor gene, which is contained within pcDNA I, were capable of growth. The transformed bacteria were then grown to confluence, and the plasmid DNAs isolated using standard techniques (e.g., see

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Ausebel et al., Current Protocols in Molecular Biology, John Wiley Sons, New York, 1989). These DNAs were then taken up in a DEAE-dextran solution, and used to transfect African Green Monkey kidney (COS) cells, which 5 had been grown to 75% confluence in "sideflasks" (Nunc, Denmark).

Screening for COS cells containing plasmids capable of expressing functionally-intact ROS or OK cell parathyroid hormone/parathyroid hormone related-protein 10 (PTH/PTH<sub>r</sub>P) receptor proteins was performed according to Gearing et al. (EMBO J. 8: 3676, 1989), with some minor modifications including DEAE-Dextran transfection in sideflasks. Forty-eight hours after transfection, the cells were tested for binding of <sup>125</sup>I-labeled [Tyr<sup>36</sup>]PTH<sub>r</sub>P 15 (1-36) amide, using methods previously described (Yamamoto et al., Endocrinology 122: 1208, 1988), with the following exceptions: the time and temperature of the incubation were 2h and room temperature, respectively. After rinsing, the cells were fixed with 1.25% 20 glutaraldehyde, and rinsed with 1% gelatin. After snapping off the top of the sideflask, the remaining microscope slide was dipped into NTB-2 photographic emulsion (Eastman Kodak, Rochester, NY). After 3-4 days of exposure at 4°C, the slides were developed, fixed, and 25 stained with 0.03% toluene blue. Screening of each slide was performed under a light microscope (Olympus). One pool of plasmid-DNA from ROS cells, and two pools of plasmid-DNA from OK cells, (10,000 independent clones), each gave rise to 3-4 transfected COS cells expressing 30 the PTH/PTH<sub>r</sub>P receptor. These pools were subsequently subdivided. The subpools were used to transfect COS cells, and single clones were identified that expressed receptor protein capable of binding the radioligand.

Isolation of cDNA and genomic DNA clones encoding  
35 the human PTH/PTH<sub>r</sub>P receptor: A human kidney oligo dT-

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primed cDNA library ( $1.7 \times 10^6$  independent clones) in lambda GT10 and a genomic library of human placental DNA ( $2.5 \times 10^6$  independent clones) in EMBL3 (Sp6/T7) (Clontech, Palo Alto, CA) were screened by the plaque hybridization technique (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed. pp. 108-113, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989) with the  $^{32}\text{P}$ -labelled (random primed labelling kit Boehringer Mannheim, Germany) BamHI/NotI 1.8kb restriction enzyme fragment encoding most of the coding sequence of the rat bone PTH/PTHrp receptor (Fig. 3). The nitrocellulose filters were incubated at  $42^\circ\text{C}$  for 4 hrs in a prehybridization solution containing 50% formamide, 4x saline sodium citrate (SSC;  $^1\text{x}$  SSC: 300 mM NaCl, 30 mM NaCitrate, pH 7.0), 2x Denhardt's solution, 10% Dextran sulphate, 100  $\mu\text{g}/\text{ml}$  salmon sperm DNA (final concentration). The hybridizations were carried out in the same solution at  $42^\circ\text{C}$  for 18-24h. Filters were washed with 2x SSC/0.1% SDS for 30 minutes at room temperature and then with  $^1\text{x}$  SSC/0.1% SDS for 30 minutes at  $45^\circ\text{C}$ . The films were exposed at  $-80^\circ\text{C}$  for 18-24h using intensifying screens.

About 1,000,000 clones were screened from each library. Positive clones were plaque-purified and lambda phage DNA was isolated (Sambrook et al., *supra*). Cloned inserts were removed from phage DNA by digestion with restriction endonucleases HindIII and EcoRI (lambda GT10 library), or with XhoI and SstI (EMBL3 library), and were then subcloned into pcDNA1 (Invitrogen, San Diego, CA) using the appropriate, dephosphorylated restriction sites. Sequencing of the  $\text{CsCl}_2$ -purified subclones was performed according to Sanger et al. (Biochem 74:5463, 1977) by the dideoxy termination method (Sequenase version 2 sequencing kit, United States Biochemical Corporation, Cleveland, OH).

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Reverse transcription and polymerase chain

reaction (PCR): 3 µg of poly (A)+ RNA from human kidney (Clontech, Palo Alto, CA) in 73.5 µl of H<sub>2</sub>O was incubated at 100°C for 30 seconds, quenched on ice, and then added 5 to 20 µl of 5x RT buffer (1x RT buffer: 40 mM Tris-HCl, pH 8.2, 40 mM KCl, 6.6 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, and dNTPs at 0.5 mM each), 2 µl (4 units) RNasin (Promega Biotec, Madison, WI), 1 µl (80 pmol/µl) of the human cDNA primer H12

10 (5'-AGATGAGGCTGTGCAGGT-3'; SEQ ID NO.: 14) and 80 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL). The reaction mixture was incubated for 40 minutes at 42°C. One-tenth of the first strand synthesis reaction mixture was then amplified by 15 PCR in a final volume of 100 µl containing 3 mM MgSO<sub>4</sub>, 200 µM dNTPs, 2 units of Vent polymerase (New England Biolab, Beverly, MA), and 2 µM each of the forward and the reverse primers (PCR conditions: denaturing for 1 min at 94°C, annealing for 1 min at 50°C, and extension at 20 72°C for 3 minutes; 40 cycles).

Two independent PCRs were performed using two different forward primers: i) degenerate primer RK-1 (5'-GGAATTCCATGGGAGCGGCCGGAT-3'; SEQ ID NO.: 15) based on

25 G CC  
the 5' coding end of the two previously cloned PTH/PTHrP receptors (described above), and ii) primer RK-2 (5'-CGGGATCCCCGGGCCCTAGGGCGGT-3'; SEQ ID NO.: 16) based on the 5' untranslated region of the human genomic clone 30 HPG1. Both PCR reactions used the reverse primer H26 (5'AGTATAGCGTCCTTGACGA-3'; SEQ ID NO.: 17) representing nucleotides 713 to 731 of the coding region of the human PTH/PTHrP receptor (Fig. 4). PCR products were blunt-ended using Klenow enzyme and cloned into 35 dephosphorylated pcDNAI cut with EcoRV.

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Northern blot analysis: Total RNA was extracted from SaOS-2 cells and from human kidney by the guanidine thiocyanate method (Chirgwin et al., Biochem. 18:5294, 1979). For Northern blot analysis, ~10 µg of total RNA 5 was subjected to electrophoresis on a 1.5%/37% formaldehyde gel and blotted onto nitrocellulose filters (Schleicher and Schuell, Keene, NH). The hybridization conditions were the same as those for screening the phage libraries (see above). The filters were washed at a final 10 stringency of 0.5x SSC/0.1% SDS for 30 min at 60°C and exposed for autoradiography.

Southern blot analysis: Human genomic DNA was prepared using the SDS/proteinase K method (Gross-Bellard et al., Eur. J. Biochem. 36:32, 1973). For Southern 15 analysis, ~10 µg of DNA was digested with SstI, PvuII and XbaI; subjected to electrophoresis on a 0.8% agarose gel; and blotted onto nitrocellulose membranes (Schleicher and Schuell, Keene, NH). The hybridization conditions were the same as those for screening the phage libraries (see 20 above). The filters were washed at a final stringency of 0.5x SSC/0.1% SDS for 30 min at 55°C and exposed for autoradiography.

#### FUNCTIONAL ASSAYS

Tests to characterize the functional properties of 25 the cloned receptors expressed on COS cells included:  
I) binding of PTH and PTHrP fragments and analogues, II) stimulation of cyclic AMP accumulation by PTH and PTHrP fragments and analogues,  
III) increase of intracellular free calcium by PTH 30 and PTHrP fragments and analogues, and  
IV) activation of inositol phosphate metabolism by PTH and PTHrP fragments and analogues. The methodologies are as follows:

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Radioreceptor Assay

[Nle<sup>8</sup>, Nle<sup>18</sup>, Tyr<sup>34</sup>]bPTH-(1-34)amide (NlePTH), and [Tyr<sup>36</sup>]PTHrP(1-36)amide(PTHrP) were iodinated with Na<sup>125</sup>I (carrier free, New England Nuclear, Boston, MA) as 5 previously reported (Segre et al., J. Biol. Chem. 254: 6980, 1979), and purified by reverse-phase HPLC. In brief, the labeled peptide was dissolved in 0.1% trifluoracetic acid (TFA), applied to a C<sub>18</sub> Sep-pak cartridge (Waters Associates, Inc., Milford, MA) and 10 eluted with a solution of 60% acetonitrile in 0.1% TFA. After lyophilization, the radioligand then was applied to C<sub>18</sub>-μBondapak column (3.9 mm x 30 cm. Waters Associates) and eluted over 30 min with a linear gradient of 30-50% acetonitrile-0.1% TFA at a flow rate of 2 ml/min. The 15 radioligand eluted in two peaks; the first peak, which eluted at approximately 38% acetonitrile, was used in these studies because it gave higher total and specific . bindings. The specific activity was 500 ± 75 mCi/mg, which corresponds to an average iodine-peptide ratio of 20 1.

COS-7 cells were grown in 15 cm plates in DMEM, 10% heat-inactivated FBS, 10 mg/L gentamycin until 80-90% confluent. Twenty-four hours after transfection by the

25 DEAE/Dextran method (Sambrook et al., *supra*), with 1-2 μg of plasmid DNA, the cells were trypsinized and replated in multiwell plastic dishes (16 or 35 mm diameter, Costar, Cambridge, MA) at a cell concentration of 5 x 10<sup>4</sup> cells/cm<sup>2</sup>). Cell number increased only slightly after 30 transfection. After continuing culture for another 48 h, radioreceptor assays were performed. The culture medium was replaced with buffer containing 50 mM Tris-HCL (pH 7.7), 100 mM NaCl, 2 mM CaCl<sub>2</sub>, 5 mM KCL, 0.5% heat-inactivated 35 fetal bovine serum (GIBCO), and 5% heat-inactivated horse

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serum (KC Biological Inc., Lenexa, KS) immediately before studies were initiated. Unless otherwise indicated, studies were conducted with cells incubated in this buffer at 15°C for 4 h with  $4 \times 10^5$  cpm/ml ( $9.6 \times 10^{-11}$  M) 5 of  $^{125}\text{I}$ -labeled NlePTH or PTHrP.

Incubations were terminated by aspirating the buffer, and repeatedly (x3) washing the culture dishes containing the adherent cells with chilled 0.9% NaCl solution, over a 15 sec period. Cell-bound radioactivity 10 was recovered by the sequential addition (x3) of 1 N NaOH (200  $\mu\text{l}$ ) to each well. After 30 min at room temperature, the NaOH was transferred to a glass tube. A second and third extraction with 1 N NaOH (200  $\mu\text{l}$ ) were combined with the first, and the total radioactivity was 15 counted in a  $\gamma$ -spectrometer (Packard Instruments, Downers Grove, IL). Tracer adherence to culture vessel without cells was negligible (<0.2% of total counts added), if vessels were preincubated with culture medium.

Determinations of cAMP accumulation

20 Intracellular cAMP accumulation was measured as described previously (Abou-Samra et al., J. Biol. Chem. 262:1129, 1986). Cells in 24-well plates were rinsed with culture medium containing 0.1% BSA and 2mM IBMX. The cells were then incubated with PTH or PTHrP for 15 25 min. at 37° C. The supernatant was removed and the cells immediately frozen by placing the whole plate in dry ice powder. Intracellular cAMP was extracted by thawing the cells in 1ml of 50 mM HCl and analyzed by a specific radioimmunoassay using an anti-cAMP antibody (e.g., 30 Sigma, St. Louis, MO). A cAMP analog (2'-O-monosuccinyl-adenosine 3':5'-cyclic monophosphate tyrosyl methyl ester, obtained from Sigma) which was used a tracer for cAMP was iodinated by the chloramine T method. Free iodine was removed by adsorbing the iodinated cAMP analog 35 onto a C18 Sep-pak cartridge (Waters, Milford, MA).

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After washing with dH<sub>2</sub>O, the iodinated cAMP analog was eluted from the Sep-pak Cartridge with 40% acetonitrille (ACN) and 0.1% trifluoroacetic acid (TFA). The iodinated cAMP analog was lyophilized, reconstituted in 1 ml 0.1% TFA, and injected into a C18 reverse phase HPLC column (Waters). The column was equilibrated with 10% ACN in 0.1% TFA, and eluted with gradient of 10-30% ACN in 0.1% TFA. This allows separation of the mono-iodinated cAMP analog from the non-iodinated cAMP analog. The tracer is 10 stable for up to 4 months when stored at -20° C. The standard used for the assay, adenosine 3':5'-cyclic monophosphate, was purchased from Sigma. Samples (1-10 µl of HCl extracts) or standards (0.04-100 fmol/tube) were diluted in 50 mM Na-acetate (pH 5.5), and acetylated 15 with 10 µl of mixture of triethylamine and acetic anhydride (2:1 vol:vol). After acetylation, cAMP antiserum (100 µl) was added from a stock solution (1:4000) made in PBS (pH 7.4), 5 mM EDTA and 1% normal rabbit serum. The tracer was diluted in PBS (pH 7.4) 20 with 0.1% BSA, and added (20,000 cpm/tube). The assay was incubated at 4° C overnight. The bound tracer was precipitated by adding 100 µl of goat anti-rabbit antiserum (1:20 in PBS) and 1 ml of 7% polyethyleneglycol (MW 5000-6000), centrifuging at 2000 rpm for 30 min. at 25 4° C. The supernatant was removed and the bound radioactivity was counted in a  $\gamma$ -counter (Micromedic). Standard curves were calculated using the four-parameter RIA program supplied by Micromedic. Typically, the assay sensitivity is 0.1 fmol/ tube, and the standard 30 concentration that displaces 50% of tracer is 5 fmol/tube.

In an alternative method for assaying cAMP accumulation, COS cells transfected with PTH/PTHrP receptor cDNA are harvested with a plastic policeman into 35 a solution containing 10 mM Tris-HCl (pH 7.5), 0.2 mM

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MgCl<sub>2</sub>, 0.5 mM ethyleneglycolbis(β-amino ethyl ether) N,N'-tetra-acetic acid (EGTA) (Sigma) and 1 mM dithiothreitol (Sigma). Cells are homogenated by 20 strokes of tightly-fitting Dounce homogenizer, and centrifuged at 13,000 × g  
5 for 15 min at 4°C (Eppendorf, type 5412, Brinkmann Instruments, Inc., Westburg, NY). The pellet containing the plasma membranes is resuspended in the same buffer by several strokes with a Dounce homogenizer, and further diluted with the same buffer to a protein concentration  
10 of approximately 1.2 mg/ml, as determined by the method of Lowry et al. (Lowry et al., J. Biol. Chem. 193: 265, 1951). Approximately 30 µg (25 µl) membrane are incubated with varying concentrations of hormone or vehicle alone for 10 min at 37°C (final volume, 100 µl)  
15 in 50 mM Tris-HCl (pH 7.5), 0.8 mM ATP, 4 × 10<sup>6</sup> cpm [ $\alpha$ -<sup>32</sup>P] ATP (New England Nuclear, Boston, MA), 9 mM theophylline, 4.2 mM MgCl<sub>2</sub>, 26 mM KCl, 0.12% BSA, and an ATP-regenerating system containing 5 mM creatine phosphate (Schwartz/Mann Division, Becton-Dickenson &  
20 Co., Orangeburg, NY) and 0.1 mg/ml creatine phosphokinase (Shwartz/Mann). Incubations are initiated by addition of the membrane suspension and terminated by addition of 100 µl of a solution containing 20 mM cAMP, approximately 50,000 cpm [<sup>3</sup>H]cAMP, and 80 mM ATP. The reaction mixture  
25 is boiled, and the [<sup>32</sup>P]cAMP generated is purified by sequential chromatography on ion-exchange columns (Dowex 50 W-X4, Biorad Lab, Richmond, CA) and alumina (Sigma). The [<sup>32</sup>P]cAMP may be counted in a β-scintillation counter (Packard Instrument Co.), with correction for recovery of  
30 [<sup>3</sup>H]cAMP.

Determination of intracellular free calcium

Measurements of intracellular calcium levels in cells transfected with PTH/PTHrP receptor cDNAs were performed using Fura-2 AM (acetomethoxy ester of Fura-2,

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Molecular Probes Inc., Eugene, OR) loaded cells. Details of the methodology are:

Coverslips plated with COS cells were incubated in Fura-2 AM loading buffer containing, in mM: HEPES (N-[2-Hydroxyethyl]piperazine-N'-(2-ethanesulfonic acid]), 5; CaCl<sub>2</sub>, 1; KCl, 5; NaCl, 145; MgSO<sub>4</sub>, 0.5; NaHCO<sub>3</sub>, 25; K<sub>2</sub>HPO<sub>4</sub>, 1.4; glucose, 10; and Fura-2 AM 91-(2-5'-carboxyoxazol-2'-yl)-6-aminobenzofuran-5oxy-(2'-amino-5'-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid 10 acetomethoxy ester), 0.5; at 37°C at pH7.4, aerated with 95% air and 5% CO<sub>2</sub> for 45 minutes. Cells loaded with Fura-2 AM were then washed with a modified Krebs-Heinseleit (KH) buffer containing, in mM: HEPES, 20; CaCl<sub>2</sub>, 1; KCl, 5; NaCl, 145; MgSO<sub>4</sub>, 0.5; Na<sub>2</sub>HPO<sub>4</sub>, 1; 15 glucose, 5; pH7.4. To check that cleavage of the ester occurred, the excitation spectra after different times of Fura-2 AM incubation were measured. At 5 min. after the start of incubation, the excitation spectrum peaked at approximately 360 nm, reflecting incomplete hydrolysis of 20 Fura-2 AM, whereas beyond 30 min. the excitation spectrum peaked at 345 nM, characteristic of Fura-2.

To measure fluorescence of individual cells, the cover slips were placed in a microscope tissue chamber (Biophysica Technologies, Inc., MD). The chamber 25 consisted of a shallow, sloped compartment made of Teflon with a silicone rubber seal. The cover slips served as the bottom of the chamber. A heater/cooler ring was encased in the silicone rubber which sealed the coverslip in place. Temperatures were varied between 22°C and 37°C 30 by applying 0-7.4 V to the heater. If the temperature is not specifically stated, the experiment was performed at 37°C. The chamber was mounted on the stage of an inverted microscope (Zeiss IM-35, Thornwood, NY). Fura-2 fluorescence was excited with a 75 watt Xenon arc lamp 35 placed at the focal point of a condenser (Photon

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Technologies International (PTI) Inc., NJ). Grating monochromators, alternated by a rotating chopper in which mirror vanes alternate with transmitting sectors, were used for selecting wavelengths. The monochromator 5 outputs were combined to form a common optical path which exited the source housing through an adjustable iris. The light then passed through quartz lenses and a dichroic mirror through a 100x Nikon Fluor objective. A photon-counting PMT device detection was used to measure 10 the light output. Data analysis was performed using PTI software run on an IBM-compatible AT/286 computer using the MS-DOS operating system. Data was retained and manipulated in a packed binary format.

Intracellular calcium concentrations were 15 calculated according to the formula:  $[Ca^{2+}]_i = K_d(R - R_{min}) / (R_{max} - R)B$ , where R is the ratio of fluorescence of the cell at 340 and 380 nm; R<sub>max</sub> and R<sub>min</sub> represent the ratios of Fura-2 fluorescence intensity at 340 and 380 nm excitation wavelengths in the presence of a saturating 20 amount of calcium and effectively zero calcium, respectively; B is the ratio of fluorescence of Fura-2 at 380 nm in zero calcium to that in saturating amounts of calcium; and K<sub>d</sub> is the dissociation constant of Fura-2 for calcium. To determine R<sub>max</sub>, at the end of an 25 experiment ionomycin was added to the Fura-2 AM loaded cells to equilibrate Ca<sup>2+</sup> between the extracellular (1mM) and intracellular environments. To calculate R<sub>min</sub>, 1mM EGTA was then added to the bathing solution. Different dissociation constants were used at the different 30 temperatures: 224 nM at 34-37°C and 135 nM at 24-27°C.

Determination of inositol phosphate

The level of inositol phosphate metabolism was determined in COS cells transfected with PTH/PTHrP

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receptors using previously published methods (Bonventre, et al., J. Biol. Chem. 265: 4934, 1990).

#### RESULTS

##### Molecular characterization

5 Two independent clones (OK-H and OK-O), both of which were isolated from the OK cell cDNA library, had lengths of approximately 2 kilobases. The determined nucleotide sequence and predicted amino acid sequence of these clones are shown in Figs. 1 (SEQ ID NO.:1) and 2  
10 (SEQ ID NO.:2) respectively. The R15B clone isolated from the ROS cell cDNA library had a length of approximately 4 kilobases. The determined nucleotide sequence and predicted amino acid sequence of the rat bone PTH/PTHrP receptor is depicted in Fig. 3 (SEQ ID  
15 NO.:3).

The three cDNA clones appear to be full-length by the criteria of having codons encoding methionine residues that are predicted to be the likely candidates as initiator methionines. These methionine codons are  
20 followed by amino acid sequences (deduced from the DNA) with properties suggesting that they are "signal-peptide" sequences. All three receptor cDNAs have stop codons at locations that permit these receptors to "fit" a putative seven-membrane spanning model, a model typical for G-  
25 protein-linked receptors. Most importantly, all three cloned receptors bind ligands and, when activated, are capable of activating intracellular effectors. These properties suggest that all three of the isolated clones encode full-length cDNAs.

30 Fig. 4 demonstrates the high degree of homology between the amino acid sequences encoded by the cDNAs from OK-O and ROS 15B. There is an overall 87% homology and a 77.8% amino acid identity between these two receptors. This high level of identity over long

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stretches of amino acids demonstrates that the amino acid sequence of the PTH receptor is evolutionarily conserved to a high degree. This allows the data from both OK-O and R15B to be extrapolated to other species, including  
5 human.

Fig. 5 shows the deduced amino acid sequences of all three cloned cDNAs lined up according to sequence homology. The OK-H sequence is identical to OK-O except in the C-terminus tail, where the OK-O sequence totals  
10 585 amino acids whereas the OK-H sequence stops at 515 amino acids. This difference is attributable to a single nucleotide (G) deleted in the OK-H sequence compared to the OK-O sequence, causing a frame shift and early stop codon in the former. It is not known whether OK-O and  
15 OK-H represent the products of two separate genes or of a laboratory artifact.

Some G-protein-coupled receptors are encoded by intronless genes (Kobilka et al., Nature 329:75, 1987); Kobilka et al., J. Biol. Chem. 262:7321, 1987; Heckert et  
20 al., Mol. Endocrinol. 6:70, 1992; Kobilka et al., Science 238:650, 1987; Bonner et al., Science 237:527, 1987; Sunahara et al., Nature 347:80, 1990). To isolate a human PTH/PTHrP receptor cDNA, both a human cDNA library and a human genomic library were screened with a probe  
25 (BamHI/NotI) representing most of the coding region of the rat bone PTH/PTHrP receptor (Fig. 3). Screening the human kidney cDNA library led to the isolation of the clone HK-1 (Fig. 6) [SEQ ID NO.: 6]. Since one of the two EcoRI cloning sites of lambda GT10 proved to be  
30 eliminated as a result of the library construction, the HindIII/EcoRI phage fragment containing the cDNA insert and ~250 bp of the 37 kb (left) lambda arm was subcloned into the corresponding restriction sites in pcDNAI. DNA sequencing revealed that the cloned cDNA contained ~1000  
35 bp of the 3' coding region and ~200 bp of the 3' non-

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coding region including an A-rich 3' end. The coding region 5' to the XhoI site was subsequently used to re-screen the library and led to the isolation of the clone HK-2 which, after subcloning into pcDNAI, proved to 5 contain ~1400 bp of the coding region. For the third screening of the library, the PvuII/PstI fragment of HK-2 was used; the isolated clone HK-3 proved to be identical to HK-2.

The genomic library screening (~10<sup>6</sup> pfu) resulted 10 in the isolation of four independent clones. Comparison of Southern blot analyses of restriction enzyme digests of these clones with that of normal genomic DNA, revealed that one 15 kb genomic clone, HPG1 (also referred to as HG4A), contained a SstI/SstI fragment that had the same 15 size as one hybridizing DNA species from normal human genomic DNA digested with SstI (see below). The hybridizing 2.3 kb SstI/SstI DNA fragment and an ~8 kb XhoI fragment which comprised the SstI/SstI fragment were both subcloned into pcDNAI. Further Southern blot 20 analysis of the SstI/SstI DNA fragment revealed that an ~1000 bp BamHI/SstI fragment encoded a portion of the human PTH/PTHrP receptor which later proved to represent the exon encoding the putative signal peptide and the 5' non-translated region which is interrupted by an ~1000 bp 25 intron (Fig. 7).

To isolate the remaining ~450 nucleotides of the coding region, poly (A)+ RNA from human kidney was reverse transcribed after priming with H12 (Fig. 7). After single strand synthesis, two independent PCRs were 30 performed using two different forward primers: i) a degenerate primer RK- 1 based on the 5' coding end of the two previously cloned PTH/PTHrP receptors, OK-O and R15B; and ii) primer RK-2 based on the 5' non-coding region of HPG1. H-26 was used as the reverse primer for both 35 reactions. Southern blot and restriction map analyses

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confirmed the expected size of the amplified DNA encoding the human PTH/PTHrP receptor. The blunt-ended PCR products encoding the 5' end of the human PTH/PTHrP were cloned into pcDNAI using the dephosphorylated EcoRV sites. Sequence analysis of each PCR clone confirmed their 5' nucleotide difference due to the difference in forward primer sequence, but revealed otherwise identical sequences. Nucleotide sequencing of both strands of the human PTH/PTHrP receptor cDNA revealed an open reading frame encoding a 593-amino acid protein (Fig. 6, SEQ ID NO.:4).

The full-length human kidney PTH/PTHrP receptor cDNA, HKrk, was constructed using the BamHI/PvuII fragment of PCR clone #2 and HK-2. Using the full-length cDNA encoding the human PTH/PTHrP receptor, Northern blot analysis of total RNA (~10 µg/lane) from human kidney and SaOS-2 cells revealed one major hybridizing DNA species of ~2.5 kb (Fig. 19). The XbaI digest of normal human genomic DNA, when probed with the same full-length cDNA (Fig. 20), revealed one major hybridizing species of about 5.5 kb, and two DNA species of 4 and 8 kb which weakly hybridized. These data suggest that the human PTH/PTHrP receptor is the product of a single gene. This full-length clone was then transiently expressed in COS-7 cells for functional and biological characterization by the methods cited above.

Comparison of the human receptor with the opossum kidney PTH/PTHrP receptor and the rat bone PTH/PTHrP receptor, revealed 81% and 91% amino acid sequence identity, respectively, and consequently a very similar hydrophobicity plot (Fig. 8). All extracellular cysteines including the two cysteine residues in the presumed signal peptide are conserved, as are all potential, extracellular

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N-glycosylation sites. A number of the amino acids which were not identical between the human kidney and rat bone PTH/PTHr receptors were found to be conserved between the human and the opossum receptors. These conserved amino acids include an Arg to Leu at 51, an Arg to Trp at 58, an Arg to His at 262, an Asp to His at 358, an Ile to Thr at 422, and a Thr to Leu at 427.

Biological Characterization

Functional characterization of the biological properties of the opossum and rat PTH/PTHrP receptors was performed in transiently transfected COS cells by a radioreceptor assay technique using both  $^{125}\text{I}$ -PTHrP and  $^{125}\text{I}$ -NlePTH as radioligands, and by bioassays that measure ligand-stimulated cAMP accumulation, increase in intracellular free calcium, and stimulation of inositol phosphate metabolism, by the methods cited above.

Fig. 9 demonstrates that COS cells expressing OK-H bind  $^{125}\text{I}$ -PTHrP. These data also demonstrate that binding of PTHrP is inhibited when intact PTH (1-34) or PTH analogues which are shortened at their amino terminus (i.e. the 3-34 and 7-34 analogues, which contain Nle substitutions for methionine at positions 8 and 18 and a tyrosine substitution for phenylalanine at position 34) are used as competitors for binding. Similarly, binding of  $^{125}\text{I}$ -NlePTH to COS cells expressing OK-H was inhibited when PTHrP or PTHrP fragments were used as competitors. These data indicate that PTH and PTHrP both bind to the receptor encoded by OK-H.

Fig. 10 demonstrates that COS cells expressing OK-H increase their concentration of intracellular free calcium when exposed to NlePTH, but to a smaller extent (mean = 39 nm), or not at all, when compared to COS cells expressing OK-O or R15B receptors (Fig. 12 and Fig. 14) and stimulated with NlePTH. Unlike COS cells expressing

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OK-O or R15B, COS cells expressing OK-H do not show a detectable increase in metabolism of inositol phosphate when stimulated with NlePTH (Fig. 15).

Fig. 11 demonstrates that COS cells expressing OK-  
5 O bind  $^{125}\text{I}$ -PTHrP. These data also demonstrate that binding of PTHrP is inhibited when intact PTH (1-34) or PTH analogues which are shortened at their amino terminus (i.e. the 3-34 and 7-34 analogues, which contain Nle substitutions for methionine at positions 8 and 18 and a  
10 tyrosine substitution for phenylalanine at position 34) are used as competitors for binding. Similarly, binding of  $^{125}\text{I}$ -NlePTH to COS cells expressing OK-H was inhibited when PTHrP or PTHrP fragments were used as competitors. These data indicate that PTH and PTHrP both bind to the  
15 receptor encoded by OK-O.

Fig. 12 demonstrates that COS cells expressing OK-O increase their concentration of intracellular free calcium and their rate of inositol phosphate metabolism after stimulation with NlePTH and PTHrP (Fig. 15).

20 Fig. 13 demonstrates that COS cells expressing R15B bind  $^{125}\text{I}$ -PTHrP. These data also demonstrate that binding of PTHrP is inhibited when intact PTH (1-34) or PTH analogues which are shortened at their amino terminus (i.e. the 3-34 and 7-34 analogues, which contain Nle  
25 substitutions for methionine at positions 8 and 18 and a tyrosine substitution for phenylalanine at position 34) are used as competitors for binding. Similarly, binding of  $^{125}\text{I}$ -NlePTH to COS cells expressing OK-H was inhibited when PTHrP or PTHrP fragments were used as competitors.  
30 These data indicate that PTH and PTHrP both bind to the receptor encoded by R15B.

Fig. 14 demonstrates that COS cells expressing R15B increase their concentration of intracellular calcium to an extent similar to stimulated COS cells  
35 expressing OK-O.

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Fig. 15 demonstrates that COS cells expressing R15B or OK-O increase their rate of phosphatidyl inositol hydrolysis, as evidenced by the rapid increase in inositol trisphosphate ( $IP_3$ ) and inositol bisphosphate ( $IP_2$ ) accumulation after stimulation of the cells with NlePTH or PTHrP. Conversely, COS cells expressing OK-H did not show any detectable increase in inositol trisphosphate and inositol bisphosphate accumulation after stimulation with NlePTH or PTHrP. These data suggest that the PTH receptor encoded by R15B and OK-O is coupled to phospholipase C, presumably through  $G_p$ . Since the only difference between OK-O and OK-H is in the cytoplasmic C-terminal tail, these data strongly suggest that the C-terminus of the PTH receptor encoded by OK-O and R15B is involved in the activation of phospholipase C.

Fig. 16 demonstrates that COS cells expressing R15B and OK-H increase cAMP accumulation after stimulation with NlePTH. Similar results were obtained in COS cells expressing OK-O. No cAMP stimulation was detected in COS cells transfected with the cDM8 vector alone. These data suggest that PTH receptor coupling to adenylate cyclase does not require the full length C-terminal cytoplasmic tail of the receptor.

These data demonstrate that all three PTH/PTHrP receptors cloned from both OK and ROS cell cDNA libraries bind the amino-terminal ligands of both peptides equivalently. Activation of all these receptors by ligand stimulates adenylate cyclase (as measured by increased intracellular cAMP), presumably through activation of one class of guanine nucleotide binding proteins (G-proteins). G-proteins have a trimeric peptide structure in which one of the subunits, alpha, is distinct, and the other two, beta and gamma, are identical or highly homologous. One of these G-proteins

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(G<sub>s</sub>) contains G-alpha-"stimulatory" (G-alpha-s) which is involved in the activation of adenylate cyclase.

Binding of ligand to OK-O and R15B, but not to OK-H, also increases intracellular free calcium and 5 stimulates metabolism of inositol phosphate. These properties strongly suggest that activation of both OK-O and R15B receptors by ligand results in stimulation of a second intracellular effector, phospholipase C. The coupling mechanism between these activated receptors and 10 phospholipase C is likely to be a G-protein which is distinct from G<sub>s</sub>. In contrast, the properties of the activated OK-H receptor which is truncated at the carboxy terminus, suggest that it may not activate phospholipase C, or that it activates phospholipase C inefficiently.

15 The biochemical role of the carboxy-terminal tail of the PTH/PTHrP receptor was further investigated by the construction of a carboxy-terminally-truncated rat receptor, R480, by standard PCR technology using R15B as a template and an upstream primer containing a stop codon 20 inserted at position 481. Briefly, the upstream primer was a synthetic oligonucleotide based on nucleotides 1494-1513 of the rat cDNA sequence (see Fig. 3; SEQ ID NO.: 3) to which a stop codon and an XbaI cloning site were added. Thirty PCR cycles were carried out, each 25 cycle consisting of 1 min at 92°C for denaturation, 1 min at 60°C for annealing, and 1 min at 72°C for extension. The product was cut with NsiI and XbaI and purified by gel electrophoresis. R15B was sequentially digested with XbaI and NsiI, and the purified PCR product was then 30 ligated into the XbaI-NsiI cut R15B vector. The resulting plasmid, R480, was amplified in bacteria and sequenced.

R480 encodes 480 amino acids that are identical to those in the 591 amino acids receptor. This truncated 35 cDNA was expressed in COS-7 cells (transient expression)

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and in CHO cells (stable expression). Both COS-7 and CHO cells expressing the truncated receptor, R480, and the wild type receptor, RB, bind PTH(1-34) with equivalent affinities. When activated, R480 stimulates cAMP 5 accumulation in COS7 and CHO cells as efficiently as does the wild type receptor. In contrast to the wild type receptor, R480 did not mediate any increase in  $[Ca^{2+}]_i$  when stimulated by PTH in either the COS-7 cells or the CHO cells. These data indicate that the molecular 10 requirements for activation of phospholipase C and adenylate cyclase by PTH/PTHrP receptor are distinct from each other, and point to a major role of the carboxy-terminal tail of the PTH/PTHrP receptor in coupling to phospholipase C but not to adenylate cyclase. Of course, 15 it is also possible that activated PTH/PTHrP receptors may activate additional G-proteins and/or intracellular effector molecules.

Analysis of COS-7 cells transfected with the cloned human PTH/PTHrP receptor demonstrated that 20 radiolabelled PTH(1-34) and PTHrP(1-36) (~200,000 cpm) bound to the expressed receptors with similar efficiency (specific binding:  $10.1 \pm 3.7\%$  and  $7.6 \pm 6.0\%$ , respectively) to that observed for COS-7 cells expressing R15B (specific binding:  $8.1 \pm 3.5\%$  and  $7.1 \pm 4.1\%$ , 25 respectively). The expressed human PTH/PTHrP receptors bound PTH(1-34) with 2-fold higher apparent  $K_d$  than did the rat bone PTH/PTHrP receptor: ~5 nM versus ~10 nM (Fig. 17). However, despite their high degree of amino acid homology, the two receptors showed significant 30 differences in affinity for PTH(3-34) and PTH(7-34). PTHrP(1-36) displayed a 2- to 4-fold lower affinity for the human PTH/PTHrP receptor than for the rat receptor (~35 nM for HKrk versus ~10 nM for R15B) which appeared more pronounced when PTHrP(1-36) was used as radioligand. 35 The affinities for PTH(3-34) and PTH(7-34) were 7- and

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35-fold higher with the expressed HKrK than with R15B (~7 nM versus ~45 nM for PTH(3-34), respectively; ~60 nM versus ~2000 nM for PTH(7-34), respectively). In COS-7 cells expressing either receptor, both PTH(1-34) and 5 PTHrP(1-36) stimulated the increase in intracellular free calcium and cAMP accumulation to the same extent (Fig. 18).

Relationship of PTH/PTHrP receptors

The amino acid sequence of the human PTH/PTHrP 10 receptor displays a very high degree of conservation compared to the bone PTH/PTHrP receptor from rat, a eutherian mammal, while its sequence identity with the PTH/PTHrP receptor with the opossum, a marsupial mammal, is less marked. Like the opossum kidney and the rat bone 15 receptor, the human kidney receptor induces an increase in both intra-cellular cAMP and intracellular free calcium when challenged with either PTH or PTHrP. Despite the high degree of homology between the human PTH/PTHrP receptor and the opossum and rat homologs, the 20 transiently expressed human receptor has some functional characteristics that are distinct from those of the rat bone receptor. These include a slightly higher affinity for PTH(1-34) and a significantly decreased affinity for PTHrP(1-36). Higher affinities were observed for PTH(3- 25 34) and in particular for PTH(7-34), the affinity of which for the human receptor was about 35-fold higher in comparison to the rat bone receptor. These findings may have significant implications for the future development of PTH/PTHrP analogues, since they predict that species- 30 specific tissues would be the appropriate tissues for testing the potency of antagonists (and agonists) *in vitro*.

Relationship of PTH/PTHrP receptors to other receptors

The biochemical properties of PTH and PTHrP 35 receptors suggest that they are members of the class of

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membrane receptor molecules known as G-protein-linked membrane receptors. The structural features of well-characterized G-protein receptors indicate that they all have at least seven regions of several consecutive 5 hydrophobic amino acids, each of which regions is of sufficient length to span the plasma membrane.

One subfamily of G-protein-linked membrane receptors, termed the glycopeptide receptor subfamily, includes receptors that bind and are activated by 10 glycopeptide hormones (thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, and chorionic gonadotropin). All of these receptors are characterized by (1) extensive putative amino-terminal extracellular domains (greater than 300 amino acids) that 15 are thought to contain some or all of the ligand-binding domains, and (2) considerable amino-acid homology, particularly in the seven putative transmembrane domains. A second subfamily, termed the adrenergic/muscarinic' subfamily, includes receptors that are activated by small 20 ligands, such as the catecholoamines, neuromuscular transmitters, and retinol. These receptors are all characterized by relatively short (25-75 amino acids) putative amino-terminal extracellular domains, as well as considerable amino acid homology, particularly in the 25 seven putative transmembrane domains. Activation of these receptors by their ligands appears to involve at least several of the multiple transmembrane domains, and does not appear to involve the amino-terminal portion of the receptors.

30 Several structural characteristics which can be deduced from the predicted amino acid sequence of the rat PTH/PTHrP receptor (Fig. 3) indicate that the PTH/PTHrP is a G-protein-linked receptor. The amino terminus shows characteristic features of a signal peptide, including a 35 hydrophobic domain and the presence of three consecutive

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leucine residues. This amino acid stretch of 20-28 amino acids may serve as a leader sequence, similar to the amino terminus preceding the extracellular domains of other glycoprotein receptors. There is also a cluster of 5 seven hydrophobic segments which represent putative membrane-spanning domains (Fig. 19).

The predicted amino acid sequences of the opossum kidney, rat bone and human kidney PTH/PTHrP receptors indicate that they do not fit comfortably into either of 10 these G-protein linked receptor subfamilies. Overall homology of the rat and human PTH/PTHrP receptors with the glycopeptide receptor and adrenergic/muscarinic subfamilies is approximately 10 to 20%, with a somewhat higher degree of homology within the transmembrane 15 domains. The latter is to be expected because of the limited menu of hydrophobic amino acids that could occur in those regions. Twenty percent homology is far less than that found among the receptors generally accepted to be members of each of these subfamilies. Additionally, 20 there are no portions of these sequences that have what could be characterized as intense homology (i.e., exactly matching amino acid sequences), even over limited regions.

Recent comparison with the newly characterized 25 secretin and calcitonin receptors (Ishihara et al., EMBO J 10:1635, 1991; Lin et al., Science 254:1022, 1991) has revealed between 30 and 40% identity between these receptors and the PTH/PTHrP receptor. Although the PTH/PTHrP receptor is more than 100 amino acids longer 30 than the calcitonin receptor, there is an ~32% identify between the amino acid sequences of the opossum kidney PTH/PTHrP receptor (SEQ ID NO NO.:2) and porcine kidney calcitonin receptor (GenBank accession no. M74420). A stretch of 17 out of 18 amino acids in the putative 35 transmembrane domain VII are identical. Also, two out of

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four N-linked glycosylation sites and the position of seven out of eight potentially extracellular cysteines are conserved. Major differences between the two receptors appear to lie in their NH<sub>2</sub>-terminal and COOH-terminal domains. Comparison of amino acid sequences of the rat secretin receptor (GenBank accession no. X59132) and the human PTH/PTHrP receptor indicates that there is a 43% identity between these two receptors, with a stretch of 21 out of 25 amino acids of the putative transmembrane domain VII being identical. The similarity between the PTH/PTHrP, calcitonin and secretin receptors suggests that they represent a new family of seven transmembrane-spanning G protein-coupled receptors that activate adenylate cyclase. Given the amino acid sequences of these receptors, those skilled in the art would be able to compare these sequences for regions of identity which would be useful in the design of nucleic acid probes which could then be used for the identification and isolation of other receptors which would belong to this family.

Deposit of Clones

Under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, the cDNA expression plasmids R15B, OK-O, and OK-H; the phage HPG1; and a plasmid (termed 8A6) containing part of the human clone have been deposited with the American Type Culture Collection (ATCC), where they bear the respective accession numbers ATCC No. 68571, 68572, 68573, 40998 and 68570. Applicants' assignee, The General Hospital Corporation, represents that the ATCC is a depository affording permanence of the deposits and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the

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public of the material so deposited will be irrevocably removed upon the granting of a patent. The material will be available during the pendency of the patent application to one determined by the Commissioner to be 5 entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited 10 plasmid, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent, whichever period is longer. Applicants' assignee acknowledges its responsibility to replace the deposits should the 15 depository be unable to furnish a sample when requested due to the condition of the deposit.

POLYPEPTIDES

Polypeptides according to the invention include the opossum and rat and human parathyroid hormone 20 receptors as shown in Figs. 1-3 and 6, respectively, and any other naturally-occurring receptor which can be produced by methods analogous to those used to clone and express these receptors, or by methods utilizing as a probe all or part of one of the sequences described 25 herein. In addition, any analog or fragment of a PTH receptor capable of binding to a parathyroid hormone or a parathyroid hormone-related protein is within the invention.

Specific receptor analogs of interest include 30 full-length or partial receptor proteins having an amino acid sequence which differs only by conservative amino acid substitutions: for example, substitution of one amino acid for another of the same class (e.g., valine for glycine; arginine for lysine, etc.), or by one or 35 more non-conservative amino-acid substitutions,

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deletions, or insertions located at positions which do not destroy the receptor's ability to bind to parathyroid hormone or parathyroid hormone-related protein.

Specific receptor fragments of particular interest

5 include, but are not limited to, portions of the receptor deduced to be extracellular from the primary amino acid sequence, using a hydrophobicity/hydrophilicity calculation such as the Chou-Fasman method (see, e.g., Chou and Fasman, Ann. Rev. Biochem. 47:251, 1978).

10 Hydrophilic domains, particularly ones surrounded by hydrophobic stretches (e.g., transmembrane domains) of at least 10 amino acids, present themselves as strong candidates for extracellular domains. Fig. 21 illustrates a predicted arrangement of extracellular,

15 intracellular, and transmembrane domains of one PTH receptor.

Examples of specific PTH receptor fragments include those with the following amino acid sequences (shown as standard single-letter symbols), derived from

20 the deduced amino acid sequence of the R15B clone:

Extracellular domains:

RP-1: TNETREREVFDRIGMIYTVG (SEQ ID NO.: 5)  
RP-2: VLYSGFTLDEAERLTEEEEL (SEQ ID NO.: 6)  
RP-3: VTFFFLYFLATNYYWILVEG (SEQ ID NO.: 7)  
25 RP-4: Y-RATLANTGCWDLSSGHKKWIIQVP (SEQ ID NO.: 8)  
RP-5: PYTEVSGTLWQIQMHYEM (SEQ ID NO.: 9)  
RP-6: DDVFTKEEQIFLLHRAQA (SEQ ID NO.: 10)

Intracellular domains:

RPI-7: FRRRLHCTRNY (SEQ ID NO.: 11)  
30 RPI-8: EKKYLWGFTL (SEQ ID NO.: 12)  
RPI-9: VLATKLRETNAGRCGCDTRQQYRKLLK (SEQ ID NO.: 13)  
These fragments were synthesized and purified by HPLC according to the method of Keutmann et al., (Endocrinology 117: 1230, 1984).

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EXPRESSION OF POLYPEPTIDES

Polypeptides according to the invention may be produced by expression from a recombinant nucleic acid having a sequence encoding part or all of a cell receptor of the invention, using any appropriate expression system: e.g., transformation of a suitable host cell (either prokaryotic or eukaryotic) with the recombinant nucleic acid in a suitable expression vehicle (e.g., pcDNAI). The precise host cell used is not critical to the invention; however, in the case wherein the polypeptides of the invention include all or part of the PTH/PTHrP receptor, the following host cells are preferred: COS cells, LLC-PK1 cells, OK cells, AtT20 cells, and CHO cells. The method of transfection and the choice of expression vehicle will depend on the host system selected. Mammalian cell transfection methods are described, e.g., in Ausubel et al. (Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989); expression vehicles may be chosen from those discussed, e.g., in Cloning Vectors: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987). Stably transfected cells are produced via integration of receptor DNA into the host cell chromosomes. Suitable DNAs are inserted into pcDNA, pcDNAI-Neo, or another suitable plasmid, and then cells are transfected with this plasmid with or without cotransfection with pSV-2-Neo, or pSV-2-DHFR by standard electroporation, calcium phosphate, and/or DEAE/Dextran techniques. Selection of transfected cells is performed using progressively increasing levels of G418 (Geneticin, GIBCO), and if necessary, methotrexate.

DNA sequences encoding the polypeptides of the invention can also be expressed in a prokaryotic host cell. DNA encoding a cell receptor or receptor fragment is carried on a vector operably linked to control signals capable of effecting expression in the prokaryotic host.

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If desired, the coding sequence may contain, at its 5' end, a sequence encoding any of the known signal sequences capable of effecting secretion of the expressed protein into the periplasmic space of the host cell,

5 thereby facilitating recovery of the protein and subsequent purification. Prokaryotes most frequently used are various strains of E. coli; however, other microbial strains may also be used. Plasmid vectors are used which contain replication origins, selectable

10 markers, and control sequences derived from a species compatible with the microbial host. For example, E. coli may be transformed using derivatives of pBR322, a plasmid constructed by Bolivar et al. (Gene 2: 95, 1977) using fragments derived from three naturally-occurring

15 plasmids, two isolated from species of Salmonella, and one isolated from E. coli. pBR322 contains genes from ampicillin and tetracycline resistance, and thus provides multiple selectable markers which can be either retained or destroyed in constructing the desired expression

20 vector. Commonly used prokaryotic control sequences (also referred to as "regulatory elements") are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences. Promoters commonly used to direct

25 protein expression include the beta-lactamase (penicillinase), the lactose (lac) (Chang et al., Nature 198: 1056, 1977) and the tryptophan (Trp) promoter systems (Goeddel et al., Nucl. Acids Res. 8: 4057, 1980) as well as the lambda-derived P<sub>L</sub> promoter and N-gene

30 ribosome binding site (Simatake et al., Nature 292:128, 1981).

The nature of the cell receptor proteins of the invention is such that, upon expression within a cell, it is moved to the cellular membrane and partially through

35 the membrane, so that part of it remains embedded in the

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membrane, part extends outside the cell, and part remains within the cell. Transformed cells bearing such embedded cell receptors may themselves be employed in the methods of the invention, or the receptor protein may be extracted from the membranes and purified.

5 Expression of peptide fragments lacking the hydrophobic portions of the protein responsible for anchoring the intact protein in the cellular membrane would not be expected to become embedded in the membrane; 10 whether they remain within the cell or are secreted into the extracellular medium depends upon whether or not a mechanism promoting secretion (e.g., a signal peptide) is included. If secreted, the polypeptide of the invention can be harvested from the medium; if not, the cells must 15 be broken open and the desired polypeptide isolated from the entire contents of the cells. Specific examples of polypeptides which might be expressed include, without limitation:

1) Amino-terminal portion comprising amino acids 20 1-192, including the putative leader sequence, of the rat bone PTH/PTHrP receptor as shown in Fig. 3.

2) Amino-terminal portion comprising amino acids 27-192, excluding the putative leader sequence, of the rat bone PTH/PTHrP receptor as shown in Fig. 3.

25 3) The full-length PTH/PTHrP receptor from rat bone, as shown in Fig. 3.

4) RP-1 (as described above).

5) RP-2 (as described above).

The polypeptide of the invention can be readily 30 purified using affinity chromatography. Antibodies to these polypeptides, or the receptor specific ligands, (e.g., the hormones PTH and PTHrP for the PTH/PTHrP receptor) may be covalently coupled to a solid phase support such as Sepharose 4 CNBr-activated sepharose 35 (Pharmacia), and used to separate the polypeptide of the

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invention from any contaminating substances. Typically 1 mg of ligand or antibody will be incubated with CNBr-activated sepharose at 4°C for 17-20 h (with shaking). The sepharose is rinsed with 1 M Tris HCl (pH 8) to block 5 excess active sites. The sepharose-PTH, sepharose-PTHrP, or sepharose-antibody is then incubated with the crude polypeptide in phosphate-buffered saline (pH 7.4) at 4°C for 2 h (with shaking). The sepharose is then typically packed in a column, thoroughly washed with PBS (typically 10 10 times the column volume), and eluted with dilute HCl in H<sub>2</sub>O (pH 1.85). The eluate may then be concentrated by lyophylization and its purity checked, for example, by reverse phase HPLC.

ANTI-CELL RECEPTOR ANTIBODIES

15 Cell receptor or receptor fragments of the invention may be used to generate antibodies by any conventional method well known to those skilled in the art, including those which generate polyclonal antibodies and those which generate monoclonal antibodies. For 20 example, the deduced amino acid sequence of the PTH receptor reveals a protein structure that appears to have several transmembrane (i.e., hydrophobic) domains interspersed with presumably extracellular and intracellular regions (see Fig. 21) analogous to those 25 found in other G protein-linked receptors. This information can be used to guide the selection of regions of the receptor protein which would be likely to be exposed on the cell surface, and thus would be presented to antibodies in vivo. A short peptide representing one 30 or more of such regions may be synthesized (e.g., chemically or by recombinant DNA techniques) and used to immunize an animal (e.g., a rabbit or a mouse) to generate polyclonal or monoclonal antibodies. For example, certain of the peptides of the PTH/PTHrP 35 receptor listed above (RP-1, RP-5 and RP-6) have been

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chemically synthesized using standard techniques and used to generate polyclonal antibodies in rabbits by the following procedure:

A preparation of a given peptide emulsified with 5 complete Freund's Adjuvant is injected intradermally into rabbits. Booster injections are emulsified in or complete adjuvant and injected at monthly intervals.

Antibody titer is assessed using either of two methods. First, serial dilutions of the antiserum in 1% 10 normal rabbit serum are incubated with  $^{125}\text{I}$ -labelled PTH/PTHrP receptor fragment by standard methods (e.g., see Segre et al., supra) for 24 h at 4° C. The bound  $^{125}\text{I}$ -PTH/PTHrP receptor fragments are separated from unbound by addition of 100  $\mu\text{l}$  of second antibody (anti- 15 rabbit IgG, Sigma) diluted 1:20 and 1 ml of 5% polyethylene glycol, followed by centrifugation at 2000 rpm for 30 min. at 4° C. The supernatant is removed and the pellet analyzed for radioactivity in a  $\gamma$ -counter. In the second method, cell lines expressing either native 20 (e.g., ROS 17/2.8, OK, SaOS-02 cells) or recombinant (COS cells or CHO cells transfected with R15B, OK-O or OK-H) PTH/PTHrP receptors are incubated with serially diluted antibody at 4°C, 20°C or 37°C for 1- 4 h. The cells are rinsed with PBS (x3) and incubated 25 for 2 h at 4°C with  $^{125}\text{I}$ -labelled (NEN, Dupont) or FITC- labelled (Sigma) second antibodies. After rinsing (x3 with PBS), the cells were either lysed with 0.1 M NaOH and counted in  $\gamma$ -counter (if  $^{125}\text{I}$ -labelled second antibody was used) or fixed with 1% paraformaldehyde and examined 30 by fluorescent microscopy (if FITC-labelled second antibody was used).

Another method for producing antibodies utilizes as antigen the intact cell receptor protein of the invention expressed on the surface of cells (e.g., 35 mammalian cells, such as COS cells, transfected with DNA

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encoding the receptor). Such cells are prepared by standard techniques, e.g., by the DEAE-dextran transfection method, using a vector encoding and capable of directing high-level expression of the cell receptor.

5 Such cells may be used to generate polyclonal or monoclonal antibodies. For example, monoclonal antibodies specific for the PTH/PTHrP receptor may be produced by the following procedure:

Intact COS cells expressing high levels of rat  
10 recombinant PTH receptors on the cell surface are injected intraperitoneally (IP) into Balb-c mice (Charles River Laboratories, Willmington, MA). The mice are boosted every 4 weeks by IP injection, and are hyperimmunized by an intravenous (IV) booster 3 days  
15 before fusion. Spleen cells from the mice are isolated and are fused by standard methods to myeloma cells. Hybridomas are selected in standard hypoxanthine/aminopterin/thymine (HAT) medium, according to standard methods. Hybridomas secreting antibodies  
20 which recognize the PTH receptor are initially identified by screening with cell lines which naturally express abundant copies of the PTH-receptor per cell (such as ROS17/2.8 or OK cells), using standard immunological techniques. Those hybridomas which produce antibodies  
25 capable of binding to the PTH receptor are cultured and subcloned. Secondary screening with radioreceptor and cAMP stimulation assays can then be performed to further characterize the monoclonal antibodies (see below).

SCREENING FOR PTH RECEPTOR ANTAGONISTS AND AGONISTS

30 The polypeptides and antibodies of the invention and other compounds may be screened for PTH-competition and for antagonistic or agonistic properties using the assays described herein.

In one example, those antibodies that recognize  
35 the PTH receptor on the intact cells are screened for

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their ability to compete with PTH or PTHrP for binding to a PTH/PTHrP receptor. Cells expressing PTH receptor on the cell surface are incubated with the  $^{125}\text{I}$ -PTH analog,  $^{125}\text{I}$ -NlePTH or  $^{125}\text{I}$ -PTHrP in the presence or absence of 5 the polyclonal or monoclonal antibody to be tested, for 4 h at 15°C. The antibody used may be from crude antiserum, cell medium, or ascites, or in purified form. After incubation, the cells are rinsed with binding buffer (e.g., physiological saline), lysed, and 10 quantitatively analyzed for radioactivity using a gamma-counter. Antibodies that reduce binding of the PTH analog to the PTH receptor are classified as competitive; those which do not are noncompetitive.

Compounds, including antibodies and polypeptides, 15 may be screened for their agonistic or antagonistic properties using the cAMP accumulation, intracellular calcium, and/or inositol phosphate assays described above. Cells expressing PTH receptor on the cell surface are incubated with PTH, PTH-receptor antibody, or a 20 combination of both, for 5 - 60 minutes at 37°C, in the presence of 2 mM IBMX (3-isobutyl-1-methyl-xanthine, Sigma, St. Louis, MO). Cyclic AMP accumulation is measured by specific radio-immunoassay, as described above. A compound that competes with PTH for binding to 25 the PTH receptor, and that inhibits the effect of PTH on cAMP accumulation, is considered a competitive PTH antagonist. Conversely, a compound that does not compete for PTH binding to the PTH receptor, but which still prevents PTH activation of cAMP accumulation (presumably 30 by blocking the receptor activation site) is considered a non-competitive antagonist. A compound that competes with PTH for binding to the PTH receptor, and which stimulates cAMP accumulation in the presence or absence of PTH, is a competitive agonist. A compound that does 35 not compete with PTH for binding to the PTH receptor but

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which is still capable of stimulating cAMP accumulation in the presence or absence of PTH, or which stimulates a higher accumulation than that observed by PTH alone, would be considered a non-competitive agonist.

5 USE

The polypeptides, antibodies, and other compounds of the invention are useful for the diagnosis, classification, prognosis, and/or treatment of disorders which may be characterized as related to the interaction 10 between a cell receptor of the invention and its specific ligand. For example, some forms of hypercalcemia and hypocalcemia are related to the interaction between PTH and PTHrP and the PTH/PTHrP receptor(s). Hypercalcemia is a condition in which there is an abnormal elevation 15 in serum calcium level; it is often associated with other diseases, including hyperparathyroidism, osteoporosis, carcinomas of the breast, lung and prostate, epidermoid cancers of the head and neck of the esophagus, multiple myeloma, and hypernephroma. Hypocalcemia, a condition in 20 which the serum calcium level is abnormally low, may result from a deficiency of effective PTH, e.g., following thyroid surgery.

In a first example, the compounds of the invention are used to manufacture diagnostic agents which are used 25 as diagnostic tools to diagnose hypercalcemia and to distinguish between hypercalcemic conditions, i.e., to differentiate hypercalcemia mediated by PTH or PTHrP (e.g., hyperparathyroidism and humoral hypercalcemia of malignancy), from hypercalcemia associated with diseases 30 which do not involve these factors (e.g., local osteolytic hypercalcemia mediated by the presence of metastatic tumor cells in direct contact with bone, and certain rare types of malignancy-related hypercalcemias mediated by an increase of humoral factors, such as 35 osteoclast activating factor (interleukin), lymphotoxin,

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calcitriol, type E prostaglandins, and vitamin D-like sterols).

In one method of diagnosis, serum total and/or ionized calcium levels are measured by standard 5 techniques before and after the administration of the PTH or PTHrP antagonists of the invention. PTH or PTHrP related hypercalcemias would be detectable as a decrease in serum calcium levels following administration of the antagonist of the invention. In contrast, for 10 hypercalcemic conditions mediated by factors other than PTH or PTHrP, the serum calcium levels would remain unchanged even after administration of the antagonist.

Another diagnostic application of the invention permits measurement of the level of PTH or PTHrP in a 15 biological sample in order to diagnose PTH or PTHrP related tumors, e.g., tumors which are associated with humoral hypercalcemia of malignancy, and for monitoring the levels of PTH or PTHrP during cancer therapy. This method involves assaying binding of the recombinant 20 parathyroid hormone receptor of the invention to PTH or PTHrP present in a tissue sample, using the binding assay described herein. The level of binding may be determined directly (e.g., by using radioactively labelled PTH receptor, and assaying the radioactivity bound to 25 endogenous PTH). Alternatively, binding of PTH receptor to the sample (e.g., a tissue section) may be followed by staining of the tissue sections with an antibody specific for the PTH receptor, using standard immunological techniques (Chin et al., Hybridoma 5:339, 1986).

30 In a third diagnostic approach, one could stably transfect cell lines (by the methods described in Ausubel et al., Current Protocols in Molecular Biology, Wiley Publishers, New York, 1987) with a PTH receptor gene linked to an appropriate promoter (e.g., the 35 metallothioneine promoter). Alternatively, the PTH/PTHrP

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receptor could be expressed from a eukaryotic vector, i.e., pcDNAI, and cotransfected with a mutant DHFR gene that will allow further gene amplification via methotrexate selection (Simonsen et al., Proc. Natl. 5 Acad. Sci., 80:2495-2499, 1983). Such high-level expression of the gene produces an immortal cell line which is oversensitive to PTH or PTHrP. Such cells provide a particularly useful tool for detecting serum blood levels of PTH or PTHrP. Such a cell line may be 10 used for diagnosis of conditions involving elevated PTH or PTHrP levels (e.g., those described above) or for conditions involving unusually low levels of PTH or PTHrP (e.g., those described above). Such a cell line is also useful for monitoring the regression or increase of PTH 15 or PTHrP levels during therapy for hypercalcemia or hypocalcemia, respectively.

A patient who is suspected of being hypercalcemic may be treated using the compounds of the invention. Rapid intervention is important because symptoms may 20 appear abruptly and, unless reversed, can be fatal. In one application, serum calcium levels are stabilized by an immediate course of treatment which includes antagonists of PTH or PTHrP. Such antagonists include the compounds of the invention which have been determined 25 (by the assays described herein) to interfere with PTH receptor-mediated cell activation. To administer the antagonist, the appropriate antibody or peptide (is used in the manufacture of a medicament, generally by being formulated in an appropriate carrier such as 30 physiological saline, and administered intravenously, at a dosage that provides adequate competition for PTH or PTHrP binding to the PTH receptor (e.g., a dosage sufficient to lower the serum calcium level to below 10 mg/dl). Typical dosage would be 1 ng to 10 mg of the 35 antibody or peptide per kg body weight per day.

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Treatment may be repeated as necessary for long term maintenance of acceptable calcium levels (i.e., levels < 10.1 mg/dl). This may be necessary for acute treatment of an underlying disease condition triggering  
5 hypercalcemia; or it may used, e.g., for chronic treatment of conditions such as osteoporosis.

In another application, the compounds of the invention which have been characterized, according to the methods of the invention, to be agonists are used  
10 therapeutically to treat hypocalcemia: e.g., that resulting from the partial or complete surgical removal of the parathyroid glands. Agonists may be formulated in a suitable carrier (e.g., physiological saline) and are preferably administered intravenously in a dosage that  
15 causes a rise in serum calcium to an acceptable level (i.e., approximately 8 mg/dl). A useful dosage range would be 1 ng to 10 mg of the agonist per kg body weight per day. Treatment may be repeated as necessary to maintain suitable serum calcium levels; long term  
20 treatment may be necessary for patients who have undergone parathyroid gland removal.

The nucleic acids of the invention may also be used therapeutically. Oligonucleotides which are antisense to PTH receptor mRNA (or nucleic acid  
25 constructs which express RNA that is antisense to PTH receptor mRNA) may be utilized as an anticancer therapy. This approach is useful, e.g., for hypercalcemias resulting from a genomic rearrangement or amplification which increases the amount or activity of PTH receptor,  
30 PTH or PTHrP. The method would involve introduction of the antisense oligonucleotide into the tumor cells *in vivo*. The antisense strand hybridizes with endogenous PTH receptor mRNA, interfering with translation of the protein, thereby reducing production of PTH receptor in  
35 such cells, and reducing PTH/PTHrP-associated neoplastic

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growth. Methods for antisense design and introduction into host cells are described, for example, in Weinberg et al., U.S. Patent No. 4,740,463, herein incorporated by reference. The biochemical characterization of the OK-H, OK-O and R15B PTH/PTHrP receptors of the invention demonstrate that the two transduction pathways now known to be triggered by the interaction of PTH with its receptor are distinct and may be separated. The predicted amino acid sequences of these receptors indicate that OK-H, which does not appear to activate inositol phosphate metabolism to any detectable degree, is 70 amino acids shorter at the carboxy-terminus than OK-O or R15B. By using the sequences of the invention and the information disclosed herein, one could clone and then alter (e.g. by site-directed mutagenesis) PTH/PTHrP receptor genes from any species to generate PTH/PTHrP receptors which do not activate phospholipase C. This could potentially allow the separation of different PTH-mediated actions, including bone resorption and bone formation, and could of great importance for the treatment of various bone disorders such as osteoporosis.

Nucleic acids of the invention which encode a PTH receptor may also be linked to a selected tissue-specific promoter and/or enhancer and the resultant hybrid gene introduced, by standard methods (e.g., as described by Leder et al., U.S. Patent No. 4,736,866, herein incorporated by reference), into an animal embryo at an early developmental stage (e.g., the fertilized oocyte stage), to produce a transgenic animal which expresses elevated levels of PTH receptor in selected tissues (e.g., the osteo calcin promoter for bone). Such promoters are used to direct tissue-specific expression of the PTH receptor in the transgenic animal. The form of PTH receptor utilized can be one which encodes a PTH receptor similar to that of the animal species used, or

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it can encode the PTH receptor homolog of a different species. In one particular example, transgenic chickens are engineered to express the PTH receptor from a promoter which directs high-level expression in chicken 5 oviducts. Such an animal is expected to produce eggs with higher calcium content, and thus harder shells.

Other Embodiments

Other embodiments are within the following claims. For example, the nucleic acid of the invention includes 10 genes or cDNAs or RNAs originally isolated from any vertebrate species, including birds or mammals such as marsupials, rodents, or humans. The high degree of homology demonstrated for the PTH receptors from such diverse species as opossum, rat, and human indicates that 15 the methods of isolating PTH receptors disclosed herein will be broadly applicable to the isolation of related cell receptors from a wide variety of species.

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**COMPUTER SUBMISSION OF DNA AND AMINO ACID SEQUENCES**

(1) GENERAL INFORMATION:

(i) APPLICANT: Segre, Gino V.  
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(ii) TITLE OF INVENTION: PARATHYROID HORMONE RECEPTOR AND DNA  
ENCODING SAME

(iii) NUMBER OF SEQUENCES: 3

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb storage  
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX  
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)  
(D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/681,702  
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- 51 -

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200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH:1862
- (B) TYPE:nucleic acid
- (C) STRANDEDNESS:double
- (D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO: 1:

TGGGCACAGC CACCCCTGTT GTAGTCCAGG GGCCAGCCCCA CTGAGCTGGC ATATCAGCTG	GTGGCCCCGT TGGACTCGGC CCTAGGGAAC GGCGGCG ATG GGA GCG CCC CGG ATC	Met Gly Ala Pro Arg Ile	115
		1	5
TCG CAC AGC CTT GCC TTG CTC CTC TGC TGC TCC GTG CTC AGC TCC GTC	Ser His Ser Leu Ala Leu Leu Leu Cys Cys Ser Val Leu Ser Ser Val		163
10	15	20	
TAC GCA CTG GTG GAT GCC GAT GAT GTC ATA ACG AAG GAG GAG CAG ATC	Tyr Ala Leu Val Asp Ala Asp Asp Val Ile Thr Lys Glu Glu Gln Ile		211
25	30	35	
ATT CTT CTG CGC AAT GCC CAG GCC CAG TGT GAG CAG CGC CTG AAA GAG	Ile Leu Leu Arg Asn Ala Gln Ala Gln Cys Glu Gln Arg Leu Lys Glu		259
40	45	50	
GTC CTC AGG GTC CCT GAA CTT GCT GAA TCT GCC AAA GAC TGG ATG TCA	Val Leu Arg Val Pro Glu Leu Ala Glu Ser Ala Lys Asp Trp Met Ser		307
55	60	65	70
AGG TCT GCA AAG ACA AAG AAG GAG AAA CCT GCA GAA AAG CTT TAT CCC	Arg Ser Ala Lys Thr Lys Lys Glu Lys Pro Ala Glu Lys Leu Tyr Pro		355
75	80	85	
CAG GCA GAG GAG TCC AGG GAA GTT TCT GAC AGG AGC CGG CTG CAG GAT	Gln Ala Glu Glu Ser Arg Glu Val Ser Asp Arg Ser Arg Leu Gln Asp		403
90	95	100	
GGC TTC TGC CTA CCT GAG TGG GAC AAC ATT GTG TGC TGG CCT GCT GGA	Gly Phe Cys Leu Pro Glu Trp Asp Asn Ile Val Cys Trp Pro Ala Gly		451
105	110	115	
GTG CCC GGC AAG GTG GTG GCC GTG CCC TGC CCC GAC TAC TTC TAC GAC	Val Pro Gly Lys Val Val Ala Val Pro Cys Pro Asp Tyr Phe Tyr Asp		499
120	125	130	
TTC AAC CAC AAA GGC CGA GCC TAT CGG CGC TGT GAC AGC AAT GGC AGC	Phe Asn His Lys Gly Arg Ala Tyr Arg Arg Cys Asp Ser Asn Gly Ser		547
135	140	145	150

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TGG GAG CTG GTG CCT GGG AAC AAC CGG ACA TGG GCG AAT TAC AGC GAA Trp Glu Leu Val Pro Gly Asn Asn Arg Thr Trp Ala Asn Tyr Ser Glu	155	160	165	595
TGT GTC AAG TTT CTG ACC AAC GAG ACC CGG GAA CGG GAA GTC TTT GAT Cys Val Lys Phe Leu Thr Asn Glu Thr Arg Glu Arg Glu Val Phe Asp	170	175	180	643
CGC CTC GGA ATG ATC TAC ACT GTG GGC TAC TCC ATC TCT CTG GGC TCC Arg Leu Gly Met Ile Tyr Thr Val Gly Tyr Ser Ile Ser Leu Gly Ser	185	190	195	691
CTC ACT GTG GCT GTG CTG ATT CTG GGT TAC TTT AGG AGG TTA CAT TGC Leu Thr Val Ala Val Leu Ile Leu Gly Tyr Phe Arg Arg Leu His Cys	200	205	210	739
ACC CGA AAC TAC ATT CAC ATG CAT CTC TTC GTG TCC TTT ATG CTC CGG Thr Arg Asn Tyr Ile His Met His Leu Phe Val Ser Phe Met Leu Arg	215	220	225	230
GCT GTA AGC ATC TTC ATC AAG GAT GCT GTG CTC TAC TCG GGG GTT TCC Ala Val Ser Ile Phe Ile Lys Asp Ala Val Leu Tyr Ser Gly Val Ser	235	240	245	835
ACA GAT GAA ATC GAG CGC ATC ACC GAG GAG GAG CTG AGG GCC TTC ACA Thr Asp Glu Ile Glu Arg Ile Thr Glu Glu Glu Leu Arg Ala Phe Thr	250	255	260	883
GAG CCT CCC CCT GCT GAC AAG GCG GGT TTT GTG GGC TGC AGA GTG GCG Glu Pro Pro Pro Ala Asp Lys Ala Gly Phe Val Gly Cys Arg Val Ala	265	270	275	931
GTA ACC GTC TTC CTT TAC TTC CTG ACC ACC AAC TAC TAC TGG ATC CTG Val Thr Val Phe Leu Tyr Phe Leu Thr Thr Asn Tyr Tyr Trp Ile Leu	280	285	290	979
GTG GAA GGC CTC TAC CTT CAC AGC CTC ATC TTC ATG GCT TTT TTC TCT Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser	295	300	305	1027
GAG AAA AAG TAT CTC TGG GGT TTC ACA TTA TTT GGC TGG GGC CTC CCT Glu Lys Lys Tyr Leu Trp Gly Phe Thr Leu Phe Gly Trp Gly Leu Pro	315	320	325	1075
GCC GTG TTT GTC GCT GTG TGG ACC GTG AGG GCT ACA CTG GCC AAC Ala Val Phe Val Ala Val Trp Val Thr Val Arg Ala Thr Leu Ala Asn	330	335	340	1123
ACT GAG TGC TGG GAC CTG AGT TCG GGG AAT AAG AAA TGG ATC ATA CAG Thr Glu Cys Trp Asp Leu Ser Ser Gly Asn Lys Lys Trp Ile Ile Gln	345	350	355	1171

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GTG CCC ATC CTG GCA GCT ATT GTG GTG AAC TTT ATT CTT TTT ATC AAT		1219	
Val Pro Ile Leu Ala Ala Ile Val Val Asn Phe Ile Leu Phe Ile Asn			
360	365	370	
ATA ATC AGA GTC CTG GCT ACT AAA CTC CGG GAG ACC AAT GCA GGG AGA		1267	
Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg			
375	380	385	390
TGT GAC ACG AGG CAA CAG TAT AGA AAG CTG CTG AAG TCC ACG CTA GTC		1315	
Cys Asp Thr Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Leu Val			
395	400	405	
CTC ATG CCG CTA TTT GGG GTG CAC TAC ATC GTC TTC ATG GCC ACG CCG		1363	
Leu Met Pro Leu Phe Gly Val His Tyr Ile Val Phe Met Ala Thr Pro			
410	415	420	
TAC ACA GAA GTA TCA CGG ATT CTT TGG CAA GTC CAA ATG CAC TAT GAA		1411	
Tyr Thr Glu Val Ser Gly Ile Leu Trp Gln Val Gln Met His Tyr Glu			
425	430	435	
ATG CTC TTC AAT TCA TTC CAG GGA TTT TTC GTT GCC ATT ATA TAC TGT		1459	
Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys			
440	445	450	
TTC TGC AAT GGA GAG GTA CAA GCA GAG ATC AAG AAG TCA TGG AGC CGA		1507	
Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Lys Lys Ser Trp Ser Arg			
455	460	465	470
TGG ACC CTG GCC TTG GAC TTC AAG CGG AAG GCC CGG AGT GGC AGC AGT		1555	
Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser			
475	480	485	
ACC TAC AGC TAT GGC CCC ATG GTG TCA CAT ACA AGT GTC ACC AAT GTG		1603	
Thr Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val			
490	495	500	
GGA CCT CGA GGG GGC TGG CCT TGT CCC TCA GCC CTC GAC TAGCTCCTGG		1652	
Gly Pro Arg Gly Gly Trp Pro Cys Pro Ser Ala Leu Asp			
505	510	515	
GGCTGGAGCC AGTGCCAATG GCCATCACCA GTTGCCTGGC TATGTGAAGC ATGGTTCCAT	1712		
TTCTGAGAAC TCATTGCCCTT CATCTGGCCC AGAGCCTGGC ACCAAAGATG ACGGGTATCT	1772		
CAATGGCTCT GGACTTTATG AGCCAATGGT TGGGGAACAG CCCCCCTCCAC TCCTGGAGGA	1832		
GGAGAGAGAG ACAGTCATGT GACCCATATC	1862		

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## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1863
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO: 2:

TGGGCACAGC CACCCCTGTTG GTAGTCCAGG GGCCAGCCCCA CTGAGCTGGC ATATCAGCTG	60
GTGGCCCCGT TGGACTCGGC CCTAGGGAAC GGCGGCCG ATG GGA GCG CCC CGG ATC	115
Met Gly Ala Pro Arg Ile	
1 5	
TCG CAC AGC CTT GCC TTG CTC CTC TGC TGC TCC GTG CTC AGC TCC GTC	163
Ser His Ser Leu Ala Leu Leu Cys Cys Ser Val Leu Ser Ser Val	
10 15 20	
TAC GCA CTG GTG GAT GCC GAT GAT GTC ATA ACG AAG GAG GAG CAG ATC	211
Tyr Ala Leu Val Asp Ala Asp Asp Val Ile Thr Lys Glu Glu Gln Ile	
25 30 35	
ATT CTT CTG CGC AAT GCC CAG GCC CAG TGT GAG CAG CGC CTG AAA GAG	259
Ile Leu Leu Arg Asn Ala Gln Ala Gln Cys Glu Gln Arg Leu Lys Glu	
40 45 50	
GTC CTC AGG GTC CCT GAA CTT GCT GAA TCT GCC AAA GAC TGG ATG TCA	307
Val Leu Arg Val Pro Glu Leu Ala Glu Ser Ala Lys Asp Trp Met Ser	
55 60 65 70	
AGG TCT GCA AAG ACA AAG AAG GAG AAA CCT GCA GAA AAG CTT TAT CCC	355
Arg Ser Ala Lys Thr Lys Lys Glu Lys Pro Ala Glu Lys Leu Tyr Pro	
75 80 85	
CAG GCA GAG GAG TCC AGG GAA GTT TCT GAC AGG AGC CGG CTG CAG GAT	403
Gln Ala Glu Glu Ser Arg Glu Val Ser Asp Arg Ser Arg Leu Gln Asp	
90 95 100	
GGC TTC TGC CTA CCT GAG TGG GAC AAC ATT GTG TGC TGG CCT GCT GGA	451
Gly Phe Cys Leu Pro Glu Trp Asp Asn Ile Val Cys Trp Pro Ala Gly	
105 110 115	
GTG CCC GGC AAG GTG GTG GCC GTG CCC TGC CCC GAC TAC TTC TAC GAC	499
Val Pro Gly Lys Val Val Ala Val Pro Cys Pro Asp Tyr Phe Tyr Asp	
120 125 130	

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TTC AAC CAC AAA GGC CGA GCC TAT CGG CGC TGT GAC AGC AAT GGC AGC Phe Asn His Lys Gly Arg Ala Tyr Arg Arg Cys Asp Ser Asn Gly Ser 135 140 145 150	547
TGG GAG CTG GTG CCT GGG AAC AAC CGG ACA TGG GCG AAT TAC AGC GAA Trp Glu Leu Val Pro Gly Asn Asn Arg Thr Trp Ala Asn Tyr Ser Glu 155 160 165	595
TGT GTC AAG TTT CTG ACC AAC GAG ACC CGG GAA CGG GAA GTC TTT GAT Cys Val Lys Phe Leu Thr Asn Glu Thr Arg Glu Arg Glu Val Phe Asp 170 175 180	643
CGC CTC CGA ATG ATC TAC ACT GTG GGC TAC TCC ATC TCT CTG GGC TCC Arg Leu Gly Met Ile Tyr Thr Val Gly Tyr Ser Ile Ser Leu Gly Ser 185 190 195	691
CTC ACT GTG GCT GTG CTG ATT CTG GGT TAC TTT AGG AGG TTA CAT TGC Leu Thr Val Ala Val Leu Ile Leu Gly Tyr Phe Arg Arg Leu His Cys 200 205 210	739
ACC CGA AAC TAC ATT CAC ATG CAT CTC TTC GTG TCC TTT ATG CTC CGG Thr Arg Asn Tyr Ile His Met His Leu Phe Val Ser Phe Met Leu Arg 215 220 225 230	787
GCT GTA AGC ATC TTC ATC AAG GAT GCT GTG CTC TAC TCG GGG GTT TCC Ala Val Ser Ile Phe Ile Lys Asp Ala Val Leu Tyr Ser Gly Val Ser 235 240 245	835
ACA GAT GAA ATC GAG CGC ATC ACC GAG GAG GAG CTG AGG GCC TTC ACA Thr Asp Glu Ile Glu Arg Ile Thr Glu Glu Leu Arg Ala Phe Thr 250 255 260	883
GAG CCT CCC CCT GCT GAC AAG GCG GGT TTT GTG GGC TGC AGA GTG GCG Glu Pro Pro Pro Ala Asp Lys Ala Gly Phe Val Gly Cys Arg Val Ala 265 270 275	931
GTA ACC GTC TTC CTT TAC TTC CTG ACC ACC AAC TAC TAC TGG ATC CTG Val Thr Val Phe Leu Tyr Phe Leu Thr Thr Asn Tyr Tyr Trp Ile Leu 280 285 290	979
GTG GAA GGC CTC TAC CTT CAC AGC CTC ATC TTC ATG GCT TTT TTC TCT Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser 295 300 305 310	1027
GAG AAA AAG TAT CTC TGG GGT TTC ACA TTA TTT GGC TGG GGC CTC CCT Glu Lys Lys Tyr Leu Trp Gly Phe Thr Leu Phe Gly Trp Gly Leu Pro 315 320 325	1075
GCC GTG TTT GTC GCT GTG TGG GTG ACC GTG AGG GCT ACA CTG GCC AAC Ala Val Phe Val Ala Val Trp Val Thr Val Arg Ala Thr Leu Ala Asn 330 335 340	1123

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ACT GAG TGC TGG GAC CTG AGT TCG GGG AAT AAG AAA TGG ATC ATA CAG Thr Glu Cys Trp Asp Leu Ser Ser Gly Asn Lys Lys Trp Ile Ile Gln 345 350 355	1171
G TG CCC ATC CTG GCA GCT ATT GTG GTG AAC TTT ATT CTT TTT ATC AAT Val Pro Ile Leu Ala Ala Ile Val Val Asn Phe Ile Leu Phe Ile Asn 360 365 370	1219
ATA ATC AGA GTC CTG GCT ACT AAA CTC CGG GAG ACC AAT GCA GGG AGA Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg 375 380 385 390	1267
TGT GAC ACG AGG CAA CAG TAT AGA AAG CTG CTG AAG TCC ACG CTA GTC Cys Asp Thr Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Leu Val 395 400 405	1315
CTC ATG CCG CTA TTT GGG GTG CAC TAC ATC GTC TTC ATG GCC ACG CCG Leu Met Pro Leu Phe Gly Val His Tyr Ile Val Phe Met Ala Thr Pro 410 415 420	1363
TAC ACA GAA GTA TCA GGG ATT CTT TGG CAA GTC CAA ATG CAC TAT GAA Tyr Thr Glu Val Ser Gly Ile Leu Trp Gln Val Gln Met His Tyr Glu 425 430 435	1411
ATG CTC TTC AAT TCA TTC CAG GGA TTT TTC GTT GCC ATT ATA TAC TGT Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys 440 445 450	1459
TTC TGC AAT GGA GAG GTA CAA GCA GAG ATC AAG AAG TCA TGG AGC CGA Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Lys Lys Ser Trp Ser Arg 455 460 465 470	1507
TGG ACC CTG GCC TTG GAC TTC AAG CGG AAG GCC CGG AGT GGC AGC AGT Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser 475 480 485	1555
ACC TAC AGC TAT GGC CCC ATG GTG TCA CAT ACA AGT GTC ACC AAT GTG Thr Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val 490 495 500	1603
GGA CCT CGA GGG GGG CTG GCC TTG TCC CTC AGC CCT CGA CTA GCT CCT Gly Pro Arg Gly Gly Leu Ala Leu Ser Leu Ser Pro Arg Leu Ala Pro 505 510 515	1651
GGG GCT GGA GCC AGT GCC AAT GGC CAT CAC CAG TTG CCT GGC TAT GTG Gly Ala Gly Ala Ser Ala Asn Gly His His Gln Leu Pro Gly Tyr Val 520 525 530	1699
AAG CAT GGT TCC ATT TCT GAG AAC TCA TTG CCT TCA TCT GGC CCA GAG Lys His Gly Ser Ile Ser Glu Asn Ser Leu Pro Ser Ser Gly Pro Glu 535 540 545 550	1747

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CCT GGC ACC AAA GAT GAC GGG TAT CTC AAT GGC TCT GGA CTT TAT GAG	1795
Pro Gly Thr Lys Asp Asp Gly Tyr Leu Asn Gly Ser Gly Leu Tyr Glu	
555	560
565	
CCA ATG GTT GGG GAA CAG CCC CCT CCA CTC CTG GAG GAG AGA GAG	1843
Pro Met Val Gly Glu Gln Pro Pro Pro Leu Leu Glu Glu Arg Glu	
570	575
580	
ACA GTC ATG TGACCCATAT C	1863
Thr Val Met	
585	

## (2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	2051
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	double
(D) TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO: 3:

GGCGGGGGCC GCGGCGGCCGA GCTCGGAGGC CGGCGGCCGGC TGCCCCGAGG GACGCGGCC	60
TAGGCGGTGG CG ATG GGG GCC CGG ATC GCA CCC AGC CTG GCG CTC	108
Met Gly Ala Ala Arg Ile Ala Pro Ser Leu Ala Leu	
1	5
10	
CTA CTC TGC TGC CCA GTG CTC AGC TCC GCA TAT GCG CTG GTG GAT GCG	156
Leu Leu Cys Cys Pro Val Leu Ser Ser Ala Tyr Ala Leu Val Asp Ala	
15	20
25	
GAC GAT GTC TTT ACC AAA GAG GAA CAG ATT TTC CTG CTG CAC CGT GCC	204
Asp Asp Val Phe Thr Lys Glu Glu Gln Ile Phe Leu Leu His Arg Ala	
30	35
40	
CAG GCG CAA TGT GAC AAG CTG CTC AAG GAA GTT CTG CAC ACA GCA GCC	252
Gln Ala Gln Cys Asp Lys Leu Leu Lys Glu Val Leu His Thr Ala Ala	
45	50
55	60
AAC ATA ATG GAG TCA GAC AAG GGC TGG ACA CCA GCA TCT ACG TCA GGG	300
Asn Ile Met Glu Ser Asp Lys Gly Trp Thr Pro Ala Ser Thr Ser Gly	
65	70
75	
AAG CCC AGG AAA GAG AAG GCA TCG GGA AAG TTC TAC CCT GAG TCT AAA	348
Lys Pro Arg Lys Glu Lys Ala Ser Gly Lys Phe Tyr Pro Glu Ser Lys	
80	85
90	
GAG AAC AAG GAC GTG CCC ACC GGC AGC AGG CGC AGA GGG CGT CCC TGT	396
Glu Asn Lys Asp Val Pro Thr Gly Ser Arg Arg Arg Gly Arg Pro Cys	
95	100
105	

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CTG CCC GAG TGG GAC AAC ATC GTT TGC TGG CCA TTA GGG GCA CCA GGT		444
Leu Pro Glu Trp Asp Asn Ile Val Cys Trp Pro Leu Gly Ala Pro Gly		
110	115	120
GAA GTG GTG GCA GTA CCT TGT CCC GAT TAC ATT TAT GAC TTC AAT CAC		492
Glu Val Val Ala Val Pro Cys Pro Asp Tyr Ile Tyr Asp Phe Asn His		
125	130	135
140		
AAA GGC CAT GCC TAC AGA CGC TGT GAC CGC AAT GGC AGC TGG GAG GTG		540
Lys Gly His Ala Tyr Arg Arg Cys Asp Arg Asn Gly Ser Trp Glu Val		
145	150	155
GTT CCA GGG CAC AAC CGG ACG TGG GCC AAC TAC AGC GAG TGC CTC AAG		588
Val Pro Gly His Asn Arg Thr Trp Ala Asn Tyr Ser Glu Cys Leu Lys		
160	165	170
TTC ATG ACC AAT GAG ACG CGG GAA CGG GAG GTA TTT GAC CGC CTA GGC		636
Phe Met Thr Asn Glu Thr Arg Glu Arg Glu Val Phe Asp Arg Leu Gly		
175	180	185
ATG ATC TAC ACC GTG GGA TAC TCC ATG TCT CTC GCC TCC CTC ACG GTG		684
Met Ile Tyr Thr Val Gly Tyr Ser Met Ser Leu Ala Ser Leu Thr Val		
190	195	200
GCT GTG CTC ATC CTG GCC TAT TTT AGG CGG CTG CAC TGC ACG CGC AAC		732
Ala Val Leu Ile Leu Ala Tyr Phe Arg Arg Leu His Cys Thr Arg Asn		
205	210	215
220		
TAC ATC CAC ATG CAC ATG TTC CTG TCG TTT ATG CTG CGC GCC GCG AGC		780
Tyr Ile His Met His Met Phe Leu Ser Phe Met Leu Arg Ala Ala Ser		
225	230	235
ATC TTC GTG AAG GAC GCT GTG CTC TAC TCT GGC TTC ACG CTG GAT GAG		828
Ile Phe Val Lys Asp Ala Val Leu Tyr Ser Gly Phe Thr Leu Asp Glu		
240	245	250
GCC GAG CGC CTC ACA GAG GAA GAG TTG CAC ATC ATC GCG CAG GTG CCA		876
Ala Glu Arg Leu Thr Glu Glu Leu His Ile Ile Ala Gln Val Pro		
255	260	265
CCT CCG CCG GCC GCT GCC GCA GTA GGC TAC GCT GGC TGC CGC GTG GCG		924
Pro Pro Pro Ala Ala Ala Val Gly Tyr Ala Gly Cys Arg Val Ala		
270	275	280
GTG ACC TTC CTC TAC TTC CTG GCT ACC AAC TAC TAC TGG ATT CTG		972
Val Thr Phe Phe Leu Tyr Phe Leu Ala Thr Asn Tyr Tyr Trp Ile Leu		
285	290	295
300		
GTG GAG GGG CTG TAC TTG CAC AGC CTC ATC TTC ATG GCC TTT TTC TCA		1020
Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser		
305	310	315

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GAG AAG AAG TAC CTG TGG GGC TTC ACC ATC TTT GGC TGG GGT CTA CCG Glu Lys Lys Tyr Leu Trp Gly Phe Thr Ile Phe Gly Trp Gly Leu Pro 320 325 330	1068
GCT GTC TTC GTG GCT GTG TGG GTC GGT GTC AGA GCA ACC TTG GCC AAC Ala Val Phe Val Ala Val Trp Val Gly Val Arg Ala Thr Leu Ala Asn 335 340 345	1116
ACT GGG TGC TGG GAT CTG AGC TCC GGG CAC AAG AAG TGG ATC ATC CAG Thr Gly Cys Trp Asp Leu Ser Ser Gly His Lys Lys Trp Ile Ile Gln 350 355 360	1164
GTG CCC ATC CTG GCA TCT GTT GTG CTC AAC TTC ATC CTT TTT ATC AAC Val Pro Ile Leu Ala Ser Val Val Leu Asn Phe Ile Leu Phe Ile Asn 365 370 375 380	1212
ATC ATC CGG GTG CTT GCC ACT AAG CTT CGG GAG ACC AAT GCG GGC CGG Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg 385 390 395	1260
TGT GAC ACC AGG CAG CAG TAC CGG AAG CTG CTC AGG TCC ACG TTG GTG Cys Asp Thr Arg Gln Tyr Arg Lys Leu Leu Arg Ser Thr Leu Val 400 405 410	1308
CTC GTG CCG CTC TTT GGT GTC CAC TAC ACC GTC TTC ATG GCC TTG CCG Leu Val Pro Leu Phe Gly Val His Tyr Thr Val Phe Met Ala Leu Pro 415 420 425	1356
TAC ACC GAG GTC TCA GGG ACA TTG TGG CAG ATC CAG ATG CAT TAT GAG Tyr Thr Glu Val Ser Gly Thr Leu Trp Gln Ile Gln Met His Tyr Glu 430 435 440 445	1404
ATG CTC TTC AAC TCC TTC CAG GGA TTT TTT GTT GCC ATC ATA TAC TGT Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys 450 455 460	1452
TTC TGC AAT GGT GAG GTG CAG GCA GAG ATT AGG AAG TCA TGG AGC CGC Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Arg Lys Ser Trp Ser Arg 465 470 475	1500
TGG ACA CTG GCG TTG GAC TTC AAG CGC AAA GCA CGA AGT GGG AGT AGC Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser 480 485 490	1548
AGC TAC AGC TAT GGC CCA ATG GTG TCT CAC ACG AGT GTG ACC AAT GTG Ser Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val 495 500 505	1596
GGC CCC CGT GCA GGA CTC AGC CTC CCC CTC AGC CCC CGC CTG CCT CCT Gly Pro Arg Ala Gly Leu Ser Leu Pro Leu Ser Pro Arg Leu Pro Pro 510 515 520 525	1644

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GCC ACT ACC AAT GGC CAC TCC CAG CTG CCT GGC CAT GCC AAG CCA GGG Ala Thr Thr Asn Gly His Ser Gln Leu Pro Gly His Ala Lys Pro Gly	530	535	540	1692
GCT CCA GCC ACT GAG ACT GAA ACC CTA CCA GTC ACT ATG GCG GTT CCC Ala Pro Ala Thr Glu Thr Glu Thr Leu Pro Val Thr Met Ala Val Pro	545	550	555	1740
AAG GAC GAT GGA TTC CTT AAC GGC TCC TGC TCA GGC CTG GAT GAG GAG Lys Asp Asp Gly Phe Leu Asn Gly Ser Cys Ser Gly Leu Asp Glu Glu	560	565	570	1788
GCC TCC GGG TCT GCG CGG CCG CCT CCA TTG TTG CAG GAA GGA TGG GAA Ala Ser Gly Ser Ala Arg Pro Pro Pro Leu Leu Gln Glu Gly Trp Glu	575	580	585	1836
ACA GTC ATG TGACTGGGCA CTAGGGGGCT AGACTGCTGG CCTGGGCACA Thr Val Met	590			1885
TGGACAGATG GACCAAGAAG CCAGTGTGTC GCTGGTTGTC TATTGGGAT CTGGACCAGG AAGATAACAA AAGGAAAATG GAAGTGGACG AAGCAGAGAA GAAGGAAGAG GTTTTGCAGG	1945			
AATTAATAT GTTCCCTCAAG TTGGATGATG AGGACACAAG GAAGGC	2005			
				2051

What is claimed is:

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Claims

1        1. Isolated DNA comprising a DNA sequence  
2    encoding a cell receptor of a vertebrate animal, said  
3    receptor having an amino acid sequence with at least 30%  
4    identity to the amino acid sequence shown in FIG. 3.

1        2. The isolated DNA of claim 1, wherein said  
2    DNA sequence encodes substantially all of the amino acid  
3    sequence shown in FIG. 1 (SEQ. ID NO. 1).

1        3. The isolated DNA of claim 1, wherein said  
2    DNA sequence encodes substantially all of the amino acid  
3    sequence shown in FIG. 3 (SEQ. ID NO. 3).

1        4. The isolated DNA of claim 1, said isolated  
2    DNA being (8A6), deposited with the ATCC and designated  
3    ATCC Accession No. 68570.

1        5. The isolated DNA of claim 1, wherein said  
2    DNA sequence encodes substantially all of the amino acid  
3    sequence shown in Fig. 6 (SEQ. ID. NO. 4).

1        6. The isolated DNA of claim 1, wherein said  
2    DNA sequence hybridizes to the DNA sequence shown in Fig.  
3    1 (SEQ. ID NO. 1).

1        7. The isolated DNA of claim 1, wherein said  
2    DNA sequence hybridizes to the DNA sequence shown in Fig.  
3    3 (SEQ. ID NO. 3).

1        8. The isolated DNA of claim 1, wherein said  
2    DNA sequence hybridizes to the DNA sequence shown in Fig.  
3    6 (SEQ. ID NO. 4).

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1               9. A purified preparation of a vector, said  
2   vector comprising a DNA sequence encoding a parathyroid  
3   hormone receptor.

1               10. A cell containing the isolated DNA of claim  
2   1.

1               11. The cell of claim 10, wherein said cell is  
2   capable of expressing said cell receptor from said  
3   isolated DNA.

1               12. An essentially homogenous population of  
2   cells, each of which comprises the isolated DNA of claim  
3   1.

1               13. Isolated DNA comprising a DNA sequence  
2   encoding a polypeptide capable of binding parathyroid  
3   hormone or parathyroid-hormone-related protein.

1               14. A method for producing a polypeptide, said  
2   method comprising:

3               providing a cell comprising isolated DNA  
4   encoding a parathyroid hormone receptor or a fragment  
5   thereof; and

6               culturing said cell under conditions  
7   permitting expression of a polypeptide from said DNA.

1               15. A single-stranded DNA comprising a portion  
2   of a parathyroid hormone receptor gene, said portion  
3   being at least 18 nucleotides long.

1               16. The single-stranded DNA of claim 15, wherein  
2   said portion is less than all of said parathyroid hormone  
3   receptor gene.

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1           17. The single-stranded DNA of claim 15, wherein  
2    said DNA is detectably labeled.

1           18. A single-stranded DNA comprising a portion  
2    of a parathyroid hormone receptor cDNA, said portion  
3    being at least 18 nucleotides long.

1           19. The single-stranded DNA of claim 18, wherein  
2    said DNA is antisense.

1           20. Parathyroid hormone receptor produced by  
2    expression of a recombinant DNA molecule encoding a  
3    parathyroid hormone receptor.

1           21. An essentially purified preparation of the  
2    parathyroid hormone receptor of claim 20.

1           22. An essentially purified preparation of the  
2    parathyroid receptor produced by the expression of the  
3    DNA of claim 5.

1           23. A polypeptide comprising at least six amino  
2    acids and less than the complete amino acid sequence of a  
3    parathyroid hormone receptor, said polypeptide capable of  
4    binding parathyroid hormone or parathyroid hormone-  
5    related protein.

1           24. The polypeptide of claim 23, wherein said  
2    parathyroid hormone receptor is a human parathyroid  
3    receptor.

1           25. The polypeptide of claim 23, wherein said  
2    fragment comprises  
3          (a) TNETREREVFDRLLGMIYTVG,  
4          (b) YLYSGFTLDEAERLTEEEL,

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5                   (c) VTFFLYFLATNYYWILVEG,  
6                   (d) Y-RATLANTGCWDLSSGHKKWIIQVP,  
7                   (e) PYTEYSGTLWQIQMHYEM,  
8                   (f) DDVFTKEEQIFLLHRAQA,  
9                   (g) FFRLHCTRNY,  
10                  (h) EKKYLWGFTL,  
11                  (i) VLATKLRETNAGRCRCDTRQQYRKLLK, or  
12                  (j) a fragment of (a) - (i) which is capable of  
13                  binding parathyroid hormone or parathyroid hormone-  
14                  related protein.

1                 26. A therapeutic composition comprising, in a  
2                 pharmaceutically-acceptable carrier, (a) a parathyroid  
3                 hormone receptor or (b) a polypeptide comprising a  
4                 fragment of said receptor.

1                 27. An antibody capable of forming an immune  
2                 complex with a parathyroid hormone receptor.

1                 28. A therapeutic composition comprising the  
2                 antibody of claim 27 and a pharmaceutically-acceptable  
3                 carrier.

1                 29. A method of reducing the level of calcium in  
2                 the blood of a mammal, which method comprises  
3                 administering the therapeutic composition of claim 26 to  
4                 said mammal in a dosage effective to inhibit activation  
5                 by parathyroid hormone or parathyroid hormone-related  
6                 protein of a parathyroid hormone receptor of said mammal.

1                 30. A method of reducing the level of calcium in  
2                 the blood of a mammal, which method comprises  
3                 administering the therapeutic composition of claim 28 to  
4                 said mammal in a dosage effective to inhibit activation

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5 by parathyroid hormone or parathyroid hormone-related  
6 protein of a parathyroid hormone receptor of said mammal.

1           31. A method for identifying a compound capable  
2 of competing with a parathyroid hormone for binding to a  
3 parathyroid hormone receptor, said method comprising:

4           (a) contacting the polypeptide of claim 23 with  
5 a parathyroid hormone, (i) in the presence or (ii) in the  
6 absence of a candidate compound; and

7           (b) comparing (i) the level of binding of said  
8 polypeptide to said parathyroid hormone in the presence  
9 of said candidate compound, with (ii) the level of  
10 binding of said polypeptide to said parathyroid hormone  
11 in the absence of said candidate compound; a lower level  
12 of binding in the presence of said candidate compound  
13 than in its absence indicating that said candidate  
14 compound is capable of competing with said parathyroid  
15 hormone for binding to said receptor.

1           32. A method for identifying a compound capable  
2 of competing with a parathyroid hormone-related protein  
3 for binding to a parathyroid hormone receptor, said  
4 method comprising:

5           (a) contacting the polypeptide of claim 23 with  
6 a parathyroid hormone-related protein, (i) in the  
7 presence or (ii) in the absence of a candidate compound;  
8 and

9           (b) comparing (i) the level of binding of said  
10 polypeptide to said parathyroid hormone-related protein  
11 in the presence of said candidate compound, with (ii) the  
12 level of binding of said polypeptide to said parathyroid  
13 hormone-related protein in the absence of said candidate  
14 compound; a lower level of binding in the presence of  
15 said candidate compound than in its absence indicating  
16 that said candidate compound is capable of competing with

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17 said parathyroid hormone-related protein for binding to  
18 said receptor.

1           33. A method for identifying a compound capable  
2 of competing with a parathyroid hormone for binding to a  
3 parathyroid hormone receptor, said method comprising:

4           (a) combining a parathyroid hormone with the  
5 cell of claim 11, (i) in the presence or (ii) in the  
6 absence of a candidate compound; and

7           (b) comparing (i) the level of binding of said  
8 receptor to said parathyroid hormone in the presence of  
9 said candidate compound, with (ii) the level of binding  
10 of said receptor to said parathyroid hormone in the  
11 absence of said candidate compound; a lower level of  
12 binding in the presence of said candidate compound than  
13 in its absence indicating that said candidate compound is  
14 capable of competing with said parathyroid hormone for  
15 binding to said receptor.

1           34. A compound capable of inhibiting the binding  
2 of parathyroid hormone or parathyroid hormone-related  
3 protein to a parathyroid receptor on the surface of a  
4 cell.

1           35. A therapeutic composition comprising the  
2 compound of claim 34 and a pharmaceutically-acceptable  
3 carrier.

1           36. A method for identifying a DNA sequence  
2 homologous to a parathyroid hormone receptor-encoding DNA  
3 sequence, said method comprising:

4           providing a genomic or cDNA library;  
5           contacting said library with the single-  
6 stranded DNA of claim 18, under conditions permitting

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7 hybridization between said single-stranded DNA and a  
8 homologous DNA sequence in said library; and  
9 identifying a clone from said library which  
10 hybridizes to said single-stranded DNA, said  
11 hybridization being indicative of the presence in said  
12 clone of a DNA sequence homologous to a parathyroid  
13 hormone receptor-encoding DNA sequence.

1           37. A transgenic non-human vertebrate animal  
2 bearing a transgene comprising a DNA sequence encoding  
3 parathyroid hormone receptor or a fragment thereof.

1           38. A diagnostic method comprising:  
2           (a) obtaining a first blood sample from an  
3 animal;       (b) administering the composition of claim  
4 35 to said animal;  
5           (c) obtaining a second blood sample from said  
6 animal subsequent to said administration of said  
7 composition; and  
8           (d) comparing the calcium level in said first  
9 blood sample with that in said second blood sample, a  
10 lower calcium level in said second blood sample being  
11 diagnostic for a parathyroid hormone-related condition.

12           39. The isolated DNA of claim 1, wherein said  
13 DNA sequence encodes a parathyroid hormone receptor.

1           40. The parathyroid hormone receptor of claim 20  
2 for use in therapy or diagnosis.

4           41. The polypeptide of claim 23 for use in  
5 therapy or diagnosis.

6           42. The antibody of claim 27 for use in therapy  
7 or diagnosis.

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8           43. The therapeutic composition of claim 26 for  
9       use in therapy for the inhibition of activation by  
10      parathyroid hormone or parathyroid hormone-related  
11      protein of a parathyroid hormone receptor of a mammal or  
12      for the reduction of the level of calcium in the blood of  
13      a mammal.

14           44. The therapeutic composition of claim 28 for  
15       use in therapy for the inhibition of activation by  
16      parathyroid hormone or parathyroid hormone-related  
17      protein of a parathyroid hormone receptor of a mammal or  
18      for the reduction of the level of calcium in the blood of  
19      a mammal.

20           45. The parathyroid hormone receptor of claim 20  
21       for use in the manufacture of a medicament for use in  
22       therapy for the inhibition of activation by parathyroid  
23      hormone or parathyroid hormone-related protein of a  
24      parathyroid hormone receptor of a mammal or for the  
25      reduction of the level of calcium in the blood of a  
26      mammal.

27           46. The polypeptide of claim 23 for use in the  
28       manufacture of a medicament for use in therapy for the  
29       inhibition of activation by parathyroid hormone or  
30      parathyroid hormone-related protein of a parathyroid  
31      hormone receptor of a mammal or for the reduction of the  
32      level of calcium in the blood of a mammal.

33           47. The antibody of claim 27 for use in the  
34       manufacture of a medicament for use in therapy for the  
35       inhibition of activation by parathyroid hormone or  
36      parathyroid hormone-related protein of a parathyroid  
37      hormone receptor of a mammal or for the reduction of the  
38      level of calcium in the blood of a mammal.

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39        48. A method for identifying a hypercalcemic  
40 condition in a patient which is mediated by parathyroid  
41 hormone or parathyroid hormone-related protein, the  
42 method comprising

43              (a) determining the calcium level of a first  
44 blood sample from the patient,  
45              (b) determining the calcium level of a second  
46 blood sample from the patient taken at a time subsequent  
47 after administration of the therapeutic composition of  
48 claim 26, and

49              (c) comparing the calcium levels of the two  
50 blood samples, a lower calcium level in the second blood  
51 sample being indicative of a condition related to  
52 parathyroid hormone or parathyroid hormone-related  
53 protein in the patient.

54        49. A method for identifying a hypercalcemic  
55 condition in a patient which is mediated by parathyroid  
56 hormone or parathyroid hormone-related protein, the  
57 method comprising

58              (a) determining the calcium level of a first  
59 blood sample from the patient,  
60              (b) determining the calcium level of a second  
61 blood sample from the patient taken at a subsequent time  
62 after administration of the therapeutic composition of  
63 claim 28, and

64              (c) comparing the calcium levels of the two  
65 blood samples, a lower calcium level in the second blood  
66 sample being indicative of a condition related to  
67 parathyroid hormone or parathyroid hormone-related  
68 protein in the patient.



10

FIG. 1

TGGGCACAGC CACCCCTGTTG GTAGTCCAGG GGCCAGCCCCA CTGAGCTGGC ATATCAGCTG	60
 GTGGCCCCGT TGGACTCGGC CCTAGGGAAC GGCGGGCG ATG GGA GCG CCC CGG ATC Met Gly Ala Pro Arg Ile 1 5	115
 TCG CAC AGC CTT GCC TTG CTC CTC TGC TGC TCC GTG CTC AGC TCC GTC Ser His Ser Leu Ala Leu Leu Leu Cys Cys Ser Val Leu Ser Ser Val 10 15 20	157
 TAC GCA CTG GTG GAT GCC GAT GAT GTC ATA ACG AAG GAG GAG CAG ATC Tyr Ala Leu Val Asp Ala Asp Asp Val Ile Thr Lys Glu Glu Gln Ile 25 30 35	211
 ATT CTT CTG CGC AAT GCC CAG GCC CAG TGT GAG CAG CGC CTG AAA GAG Ile Leu Leu Arg Asn Ala Gln Ala Gln Cys Glu Gln Arg Leu Lys Glu 40 45 50	259
 GTC CTC AGG GTC CCT GAA CTT GCT GAA TCT GCC AAA GAC TGG ATG TCA Val Leu Arg Val Pro Glu Leu Ala Glu Ser Ala Lys Asp Trp Met Ser 55 60 65 70	307
 AGG TCT GCA AAG ACA AAG AAG GAG AAA CCT GCA GAA AAG CTT TAT CCC Arg Ser Ala Lys Thr Lys Lys Glu Lys Pro Ala Glu Lys Leu Tyr Pro 75 80 85	355
 CAG GCA GAG GAG TCC AGG GAA GTT TCT GAC AGG AGC CGG CTG CAG GAT Gln Ala Glu Glu Ser Arg Glu Val Ser Asp Arg Ser Arg Leu Gln Asp 90 95 100	403
 GGC TTC TGC CTA CCT GAG TGG AAC AAC ATT GTG TGC TGG CCT GCT GGA Gly Phe Cys Leu Pro Glu Trp Asp Asn Ile Val Cys Trp Pro Ala Gly 105 110 115	441
 GTG CCC GGC AAG GTG GTG GCC GTG CCC TGC CCC GAC TAC TTC TAC GAC Val Pro Gly Lys Val Val Ala Val Pro Cys Pro Asp Tyr Phe Tyr Asp 120 125 130	499
 TTC AAC CAC AAA GGC CGA GCC TAT CGG CGC TGT GAC AGC AAT GGC AGC Phe Asn His Lys Gly Arg Ala Tyr Arg Arg Cys Asp Ser Asn Gly Ser 135 140 145 150	547
 TGG GAG CTG GTG CCT GGG AAC AAC CGG ACA TGG GCG AAT TAC AGC GAA Trp Glu Leu Val Pro Gly Asn Asn Arg Thr Trp Ala Asn Tyr Ser Glu 155 160 165	595
 TGT GTC AAG TTT CTG ACC AAC GAG ACC CGG GAA CGG GAA GTC TTT GAT Cys Val Lys Phe Leu Thr Asn Glu Thr Arg Glu Arg Glu Val Phe Asp 170 175 180	643

FIG. :

CGC CTC GGA ATG ATC TAC ACT GTG GGC TAC TCC ATC TCT CTG GGC TCC Arg Leu Gly Met Ile Tyr Thr Val Gly Tyr Ser Ile Ser Leu Gly Ser 185 190 195	691
CTC ACT GTG GCT GTG CTG ATT CTG GGT TAC TTT AGG AGG TTA CAT TGC Leu Thr Val Ala Val Leu Ile Leu Gly Tyr Phe Arg Arg Leu His Cys 200 205 210	739
ACC CGA AAC TAC ATT CAC ATG CAT CTC TTC GTG TCC TTT ATG CTC CGG Thr Arg Asn Tyr Ile His Met His Leu Phe Val Ser Phe Met Leu Arg 215 220 225 230	787
GCT GTA AGC ATC TTC ATC AAG GAT CCT GTG CTC TAC TCG GGG GTT TCC Ala Val Ser Ile Phe Ile Lys Asp Ala Val Leu Tyr Ser Gly Val Ser 235 240 245	835
ACA GAT GAA ATC GAG CGC ATC ACC GAG GAG GAG CTG AGG GCC TTC ACA Thr Asp Glu Ile Glu Arg Ile Thr Glu Glu Glu Leu Arg Ala Phe Thr 250 255 260	883
GAG CCT CCC CCT GCT GAC AAG GCG GGT TTT GTG GGC TGC AGA GTC GCG Glu Pro Pro Pro Ala Asp Lys Ala Gly Phe Val Gly Cys Arg Val Ala 265 270 275	931
GTA ACC GTC TTC CTT TAC TTC CTG ACC ACC AAC TAC TAC TGG ATC CTG Val Thr Val Phe Leu Tyr Phe Leu Thr Thr Asn Tyr Tyr Trp Ile Leu 280 285 290	979
GTG GAA GGC CTC TAC CTT CAC AGC CTC ATC TTC ATG GCT TTT TTC TCT Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser 295 300 305 310	1027
GAG AAA AAG TAT CTC TGG GGT TTC AGA TTA TTT GGC TGG GGC CTC CCT Glu Lys Lys Tyr Leu Trp Gly Phe Thr Leu Phe Gly Trp Gly Leu Pro 315 320 325	1075
GCC ATG TTT GTC GCT CTG TGG ATG AGG GCT ACA CTG GCC AAC Ala Ile Phe Val Ala Val Trp Val Thr Val Arg Ala Thr Leu Ala Asn 330 335 340	1123
ACT GAG TGC TGG GAC CTG AGT TCG GGG AAT AAG AAA TGG ATC ATA CAG Thr Glu Cys TTT Asp Leu Ser Ser Gly Asn Lys Lys Trp Ile Ile Gln 345 350 355	1171
GTG CCC ATC CTG GCA GCT ATT GTG GTC AAC TTT ATT CTT TTT ATC AAT Val Pro Ile Leu Ala Ala Ile Val Val Asn Phe Ile Leu Phe Ile Asn 360 365 370	1219
ATA ATC AGA GTC CTG GCT ACT AAA CTC CGG GAG ACC AAT GCA GGG AGA Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg 375 380 385 390	1267

FIG. 1

TGT GAC ACG AGG CAA CAG TAT AGA AAG CTG CTG AAG TCC ACG CTA GTC	1315
Cys Asp Thr Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Leu Val	
395 400 405	
CTC ATG CCG CTA TTT GGG GTG CAC TAC ATC GTC TTC ATG GCC ACG CCG	1353
Leu Met Pro Leu Phe Gly Val His Tyr Ile Val Phe Met Ala Thr Pro	
410 415 420	
TAC ACA GAA GTA TCA GGG ATT CTT TGG CAA GTC CAA ATG CAC TAT GAA	1411
Tyr Thr Glu Val Ser Gly Ile Leu Trp Gln Val Gln Met His Tyr Glu	
425 430 435	
ATG CTC TTC AAT TCA TTC CAG GGA TTT TTC GTT GCC ATT ATA TAC TGT	1452
Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys	
440 445 450	
TTC TGC AAT GGA GAG GTA CAA GCA GAG ATC AAG AAG TCA TGG AGC CGA	1507
Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Lys Lys Ser Trp Ser Arg	
455 460 465 470	
TGG ACC CTG GCC TTG GAC TTC AAG CGG AAG GCC CGG AGT GGC AGC AGT	1555
Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser	
475 480 485	
ACC TAC AGC TAT GGC CCC ATG GTG TCA CAT ACA AGT GTC ACC AAT GTG	1603
Thr Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val	
490 495 500	
GGA CCT CGA GGG GGC TGG CCT TGT CCC TCA GCC CTC GAC TAGCTCCTGG	1652
Gly Pro Arg Gly Gly Trp Pro Cys Pro Ser Ala Leu Asp	
505 510 515	
GGCTGGAGCC AGTGCCATG GCCATCACCA GTTGCCTGGC TATGTGAAGC ATGGTTCCAT	1710
TTCTGAGAAC TCATTGCCCTT CATCTGGCCC AGAGCCTGGC ACCAAAGATG ACGGGTATCT	1770
CAATGGCTCT GGACTTTATG AGCCATATGT TGGGGAAACAG CCCCTCCAC TCCTGGAGGA	1830
GGAGAGAGAG ACAGTCATGT GACCCATATC	1862

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FIG. 2

TGGGCACAGC CACCCCTGTTG GTAGTCCAGG GGCCAGCCCCA CTGAGCTGGC ATATCAGCTG	60
GTGGCCCCGT TGGACTCGGC CCTAGGGAAC GGCGGGCG ATG GGA GCG CCC CGG ATC Met Gly Ala Pro Arg Ile	115
1 5	
TCG CAC AGC CTT GCC TTG CTC CTC TGC TGC TCC GTG CTC AGC TCC GTC Ser His Ser Leu Ala Leu Leu Cys Cys Ser Val Leu Ser Ser Val	163
10 15 20	
TAC GCA CTG GTG GAT GCC GAT GAT GTC ATA ACG AAG GAG GAG CAG ATC Tyr Ala Leu Val Asp Ala Asp Asp Val Ile Thr Lys Glu Glu Gln Ile	211
25 30 35	
ATT CTT CTG CGC AAT GCC CAG GCC CAG TGT GAG CAG CGC CTG AAA GAG Ile Leu Leu Arg Asn Ala Gln Ala Gln Cys Glu Gln Arg Leu Lys Glu	259
40 45 50	
GTC CTC AGG GTC CCT GAA CTT GCT GAA TCT GCC AAA GAC TGG ATG TCA Val Leu Arg Val Pro Glu Leu Ala Glu Ser Ala Lys Asp Trp Met Ser	307
55 60 65 70	
AGG TCT GCA AAG ACA AAG AAG GAG AAA CCT GCA GAA AAG CTT TAT CCC Arg Ser Ala Lys Thr Lys Lys Glu Lys Pro Ala Glu Lys Leu Tyr Pro	355
75 80 85	
CAG GCA GAG GAG TCC AGG GAA GTT TCT GAC AGG AGC CGG CTG CAG GAT Gln Ala Glu Glu Ser Arg Glu Val Ser Asp Arg Ser Arg Leu Gln Asp	403
90 95 100	
GAC TTC TGC CTA CCT GAG TGG GAC AAC ATT GTG TGC TGG CCT GCT GGA Ile Phe Cys Leu Pro Glu Trp Ser Ser Ile Val Cys Trp Pro Ala Gly	451
105 110 115	
GTG CCC GGC AAG GTG GTG GCC CCT CCC TGC CCC GAC TAC TTC TAC GAC Val Pro Gly Lys Val Val Ala Val Pro Cys Pro Asp Tyr Phe Tyr Asp	499
120 125 130	
TTC AAC CAC AAA GGC CGA GCC TAT CGG CGC TGT GAC AGC AAT GGC AGC Phe Asn His Lys Gly Arg Ala Tyr Arg Arg Cys Asp Ser Asn Gly Ser	547
135 140 145 150	
TGG GAG CTG GTG CCT GGG AAC AAC CGG ACA TGG GCG AAT TAC AGC GAA Trp Glu Leu Val Pro Gly Asn Asn Arg Thr Trp Ala Asn Tyr Ser Glu	595
155 160 165	
TGT GTC AAG TTT CTG ACC AAC GAG ACC CGG GAA CGG GAA GTC TTT GAT Cys Val Lys Phe Leu Thr Asn Glu Thr Arg Glu Arg Glu Val Phe Asp	643
170 175 180	

FIG. 2

CGC CTC GGA ATG ATC TAC ACT GTG GGC TAC TCC ATC TCT CTG GGC TCC Arg Leu Gly Met Ile Tyr Thr Val Gly Tyr Ser Ile Ser Leu Gly Ser 185 190 195	691
CTC ACT GTG GCT GTG CTG ATT CTG GGT TAC TTT AGG AGG TTA CAT TGC Leu Thr Val Ala Val Leu Ile Leu Gly Tyr Phe Arg Arg Leu His Cys 200 205 210	700
ACC CGA AAC TAC ATT CAC ATG CAT CTC TTC GTG TCC TTT ATG CTC CGG Thr Arg Asn Tyr Ile His Met His Leu Phe Val Ser Phe Met Leu Arg 215 220 225 230	737
GCT GTA AGC ATC TTC ATC AAG GAT GCT GTG CTC TAC TCG GGG GTT TCC Ala Val Ser Ile Phe Ile Lys Asp Ala Val Leu Tyr Ser Gly Val Ser 235 240 245	805
ACA GAT GAA ATC GAG CGC ATC ACC GAG GAG GAG CTG AGG GCC TTC ACA Thr Asp Glu Ile Glu Arg Ile Thr Glu Glu Glu Leu Arg Ala Phe Thr 250 255 260	883
GAG CCT CCC CCT GCT GAC AAG GCG GGT TTT GTG GGC TGC AGA GTG GCG Glu Pro Pro Pro Ala Asp Lys Ala Gly Phe Val Gly Cys Arg Val Ala 265 270 275	931
GTA ACC GTC TTC CTT TAC TTC CTG ACC AAC TAC TAC TGG ATC CTG Val Thr Val Phe Leu Tyr Phe Leu Thr Thr Asn Tyr Tyr Trp Ile Leu 280 285 290	979
GTG GAA GGC CTC TAC CTT CAC AGC CTC ATC TTC ATG GCT TTT TTC TCT Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser 295 300 305 310	1027
GAG AAA AAG TAT CTC TGG GGT TTC ACA TTA TTT GGC TGG GGC CTC CCT Glu Lys Tyr Leu Trp Gly Phe Thr Leu Phe Gly Trp Gly Leu Pro 315 320 325	1073
GCC GTG TTT GTC GCT GTG TGG GTG ACC GTG AGG GCT ACA CTG GCC AAC Ala Val Phe Val Ala Val Trp Val Thr Val Arg Ala Thr Leu Ala Asn 330 335 340	1120
ACT GAG TGC TGG GAC CTG AGT TCG GGG AAT AAG AAA TGG ATC ATA CAG Thr Glu Cys Trp Asp Leu Ser Ser Gly Asn Lys Lys Trp Ile Ile Gln 345 350 355	1171
GTG CCC ATC CTG GCA GCT ATT GTG GTG AAC TTT ATT CTT TTT ATC AAT Val Pro Ile Leu Ala Ala Ile Val Val Asn Phe Ile Leu Phe Ile Asn 360 365 370	1219
ATA ATC AGA GTC CTG GCT ACT AAA CTC CGG GAG ACC AAT GCA GGG AGA Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg 375 380 385 390	1267

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FIG. 2

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TGT GAC ACG AGG CAA CAG TAT AGA AAG CTG CTG AAG TCC ACG CTA GTC Cys Asp Thr Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Leu Val 395 400 405	1315
CTC ATG CCG CTA TTT GGG GTG CAC TAC ATC GTC TTC ATG GCC ACG CCG Leu Met Pro Leu Phe Gly Val His Tyr Ile Val Phe Met Ala Thr Pro 410 415 420	1363
TAC ACA GAA GTA TCA GGG ATT CTT TGG CAA GTC CAA ATG CAC TAT GAA Tyr Thr Glu Val Ser Gly Ile Leu Trp Gln Val Gln Met His Tyr Glu 425 430 435	1411
ATG CTC TTC AAT TCA TTC CAG GGA TTT TTC GTT GCC ATT ATA TAC TGT Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys 440 445 450	1459
TTC TGC AAT GGA GAG GTA CAA GCA GAG ATC AAG AAG TCA TGG AGC CGA Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Lys Lys Ser Trp Ser Arg 455 460 465 470	1507
TGG ACC CTG GCC TTG GAC TTC AAG CGG AAG GCC CGG AGT GGC AGC ACT Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser 475 480 485	1555
ACC TAC AGC TAT GGC CCC ATG GTG TCA CAT ACA AGT GTC ACC AAT GTG Thr Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val 490 495 500	1603
GGA CCT CGA GGG GGG CTG GCC TTG TCC CTC AGC CCT CGA CTA GCT CCT Gly Pro Arg Gly Gly Leu Ala Leu Ser Leu Ser Pro Arg Leu Ala Pro 505 510 515	1651
GGG GCT GGA GCC AGT GCC AAT GGC CAT CAC CAG TTG CCT GGC TAT GTG Gly Ala Gly Ala Ser Ala Asn Gly His His Gln Leu Pro Gly Tyr Val 520 525 530	1699
AAG CAT CGT TCC ATT TCT GAG AAC TCA TTG CCT TCA TCT GGC CCA GAG Lys His Gly Ser Ile Ser Glu Asn Ser Leu Pro Ser Ser Gly Pro Glu 535 540 545 550	1747
CCT GGC ACC AAA GAT GAC GGG TAT CTC AAT GGC TCT GGA CTT TAT GAG Pro Gly Thr Lys Asp Asp Gly Tyr Leu Asn Gly Ser Gly Leu Tyr Glu 555 560 565	1795
CCA ATG GTT GGG GAA CAG CCC CCT CCA CTC CTG GAG GAG GAG AGA GAG Pro Met Val Gly Glu Gln Pro Pro Pro Leu Leu Glu Glu Glu Arg Glu 570 575 580	1843
ACA GTC ATG TGACCCATAT C Thr Val Met 585	1863

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FIG. 3

GGCGGGGGCC	GCGGCGGCCA	GCTCGGAGGC	CGGCGGCCGC	TGCCCGAGG	GACGCCGCC	60
TAGGCGGTGG CG ATG GGG GCC GCC CGG ATC GCA CCC AGC CTG GCG CTC						108
Met Gly Ala Ala Arg Ile Ala Pro Ser Leu Ala Leu						
1	5		10			
CTA CTC TGC TGC CCA GTG CTC AGC TCC GCA TAT GCG CTG GTG GAT GCG						115
Leu Leu Cys Cys Pro Val Leu Ser Ser Ala Tyr Ala Leu Val Asp Ala						
15	20		25			
GAC GAT GTC TTT ACC AAA GAG GAA CAG ATT TTC CTG CTG CAC CGT GCC						204
Asp Asp Val Phe Thr Lys Glu Glu Gln Ile Phe Leu Leu His Arg Ala						
30	35		40			
CAG GCG CAA TGT GAC AAG CTG CTC AAG GAA GTT CTG CAC ACA GCA GCC						211
Gln Ala Gln Cys Asp Lys Leu Leu Lys Glu Val Leu His Thr Ala Ala						
45	50		55			60
AAC ATA ATG GAG TCA GAC AAG GGC TGG ACA CCA GCA TCT ACG TCA GGG						300
Asn Ile Met Glu Ser Asp Lys Gly Trp Thr Pro Ala Ser Thr Ser Gly						
65	70		75			
AAG CCC AGG AAA GAG AAG GCA TCG GGA AAG TTC TAC CCT GAG TCT AAA						348
Lys Pro Arg Lys Glu Lys Ala Ser Gly Lys Phe Tyr Pro Glu Ser Lys						
80	85		90			
GAG AAC AAG GAC GTG CCC ACC GGC AGC AGG CGC AGA GGG CGT CCC TGT						396
Glu Asn Lys Asp Val Pro Thr Gly Ser Arg Arg Arg Gly Arg Pro Cys						
95	100		105			
CTG CCC GAG TGG GAC AAC ATC GTT TGC TGG CCA TTA GGG GCA CCA GGT						444
Leu Pro Glu Trp Asp Asn Ile Val Cys Trp Pro Leu Gly Ala Pro Gly						
110	115		120			
GAA GTG GTG GCA GTA CCT TGT CCC GAT TAC ATT TAT GAC TTC AAT CAC						492
Glu Val Val Ala Val Pro Cys Pro Asp Tyr Ile Tyr Asp Phe Asn His						
125	130		135			140
AAA GGC CAT GCC TAC AGA CGC TGT GAC CGC AAT GGC AGC TGG GAG GTG						540
Lys Gly His Ala Tyr Arg Arg Cys Asp Arg Asn Gly Ser Trp Glu Val						
145	150		155			
GTT CCA GGG CAC AAC CGG ACG TGG GCC AAC TAC AGC GAG TGC CTC AAG						588
Val Pro Gly His Asn Arg Thr Trp Ala Asn Tyr Ser Glu Cys Leu Lys						
160	165		170			
TTC ATG ACC AAT GAG ACG CGG GAA CGG GAG GTA TTT GAC CGC CTA GGC						636
Phe Met Thr Asn Glu Thr Arg Glu Arg Glu Val Phe Asp Arg Leu Gly						
175	180		185			
ATG ATC TAC ACC GTG GGA TAC TCC RTG TCT CTC GCC TCC CTC ACG GTG						684
Met Ile Tyr Thr Val Gly Tyr Ser Met Ser Leu Ala Ser Leu Thr Val						
190	195		200			

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FIG. 3

GCT GTG CTC ATC CTG GCC TAT TTT AGG CGG CTG CAC TGC ACG CGC AAC · Ala Val Leu Ile Leu Ala Tyr Phe Arg Arg Leu His Cys Thr Arg Asn 205 210 215 220	732
, TAC ATC CAC ATG CAC ATG TTC CTG TCG TTT ATG CTG CGC GCC GCG AGC Tyr Ile His Met His Met Phe Leu Ser Phe Met Leu Arg Ala Ala Ser 225 230 235	780
ATC TTC GTG AAG GAC GCT GTG CTC TAC TCT GGC TTC ACG CTG GAT GAG Ile Phe Val Lys Asp Ala Val Leu Tyr Ser Gly Phe Thr Leu Asp Glu 240 245 250	828
, GCC GAG CGC CTC ACA GAG GAA GAG TTG CAC ATC ATC GCG CAG GTG CCA Ala Glu Arg Leu Thr Glu Glu Glu Ile His Ile Ala Gln Val Pro 255 260 265	876
CCT CCG CCG GCC GCT GCC GCC GTA GGC TAC GCT GGC TGC CGC GTG GCG Pro Pro Pro Ala Ala Ala Val Gly Tyr Ala Gly Cys Arg Val Ala 270 275 280	924
GTG ACC TTC TTC CTC TAC TTC CTG CCT ACC AAC TAC TAC TGG ATT CTG Val Thr Phe Phe Leu Tyr Phe Leu Ala Thr Asn Tyr Tyr Trp Ile Leu 285 290 295 300	972
GTG GAG GGG CTG TAC TTG CAC AGC CTC ATC TTC ATG GCC TTT TTC TCA Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser 305 310 315	1020
GAG AAG AAG TAC CTG TGG GGC TTC ACC ATC TTT GGC TGG GGT CTA CCG Glu Lys Lys Tyr Leu Trp Gly Phe Thr Ile Phe Gly Trp Gly Leu Pro 320 325 330	1068
GCT GTC TTC CTG CCT GTG TGG GTC CCT GTC AGA GCA ACC TTG GCC AAC Ala Val Phe Val Ala Val Trp Val Glu Val Arg Ala Thr Leu Ala Asn 335 340 345	1116
ACT GGG TGC TGG GAT CTG AGC TCC AGG AAC AAG AAG TGG ATC ATC CAG Thr Gly Cys Trp Asp Leu Ser Ser Glu His Lys Lys Trp Ile Ile Gln 350 355 360 365	1164
GTG CCC ATC CTG GCA TCT GTG CTC AAC TTC ATC CTT TTT ATC AAC Val Pro Ile Leu Ala Ser Val Val Leu Asn Phe Ile Leu Phe Ile Asn 370 375 380	1212
ATC ATC CGG GTG CTT GCC ACT AAG CTT CGG GAG ACC AAT GCG GGC CGG Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg 385 390 395	1260
TGT GAC ACC AGG CAG CAG TAC CGG AAG CTG CTC AGG TCC ACG TTG GTG Cys Asp Thr Arg Gln Gln Tyr Arg Lys Leu Leu Arg Ser Thr Leu Val 400 405 410	1308

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## FIG. 3

CTC GTG CCG CTC TTT GGT GTC CAC TAC ACC GTC TTC ATG GCC TTG CCG Leu Val Pro Leu Phe Gly Val His Tyr Thr Val Phe Met Ala Leu Pro 415 420 425	1356
TAC ACC GAG GTC TCA GGG ACA TTG TGG CAG ATC CAG ATG CAT TAT GAG Tyr Thr Glu Val Ser Gly Thr Leu Trp Gln Ile Gln Met His Tyr Glu 430 435 440 445	1401
ATG CTC TTC AAC TCC TTC CAG GGA TTT TTT GTT GCC ATC ATA TAC TGT Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys 450 455 460	1452
TTC TGC AAT GGT GAG GTG CAG GCA GAG ATT AGG AAG TCA TGG AGC CGC Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Arg Lys Ser Trp Ser Arg 465 470 475	1502
TGG ACA CTG GCG TTG GAC TTC AAG CGC AAA GCA CGA AGT GGG AGT AGC Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser 480 485 490	1548
AGC TAC AGC TAT GGC CCA ATG GTG TCT CAC ACG AGT GTG ACC AAT GTG Ser Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val 495 500 505	1596
GCG CCC CGT GCA GGA CTC AGC CTC CCC CTC AGC CCC CGC CTG CCT CCT Gly Pro Arg Ala Gly Leu Ser Leu Pro Leu Ser Pro Arg Leu Pro Pro 510 515 520 525	1644
GCC ACT ACC AAT GGC CAC TCC CAG CTG CCT GGC CAT GCC AAG CCA GGG Ala Thr Thr Asn Gly His Ser Gln Leu Pro Gly His Ala Lys Pro Gly 530 535 540	1692
GCT CCA GCC ACT GAG ACT GAA ACC CTA CCA GTC ACT ATG GCG GTT CCC Ala Pro Ala Thr Glu Thr Glu Thr Leu Pro Val Thr Met Ala Val Pro 545 550 555	1740
AAG GAC GAT GGA TTC CTT AAC GGC TCC TGC TCA GGC CTG GAT GAG GAG Lys Asp Asp Gly Phe Leu Asn Gly Ser Cys Ser Gly Leu Asp Glu Glu 560 565 570	1783
GCC TCC GGG TCT GCG CGG CCG CCT CCA TTG TTG CAG GAA GGA TGG GAA Ala Ser Gly Ser Ala Arg Pro Pro Pro Leu Leu Gln Glu Gly Trp Glu 575 580 585	1836
ACA GTC ATG TGACTGGCA CTAGGGGGCT AGACTGCTGG CCTGGGCACA 1885 Thr Val Met 590	
TGGACAGATG GACCAAGAAG CCAGTGTTTG GCTGGTTGTC TATTCCGGAT CTGGACCAGG 1945	
AAGATAACAA AAGGAAAATG GAAGTGGACG AAGCAGAGAA GAAGGAAAGAG GTTTTGCAGG 2005	
AATTAAATAT GTTTCCCTCAG TTGGATGATG AGGACACAAG GAAGGC 2051	

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Fig. 4

1 MGAARIAPSALLLCCPVLSAYALVDAADDVFTREZQIFILL8RAOAQCDK 50  
 1 MGAPRISHSLALLLCCSVLSVYALVDAADDVITREZQIILLRNAQAQCEQ 50  
 51 LLKEVLHTAANIRESDKGWTPASTSGKPRREXASGRKFYPESKENKDVTG 100  
 51 RLKEVLR.VPELAESAKDW..MRSAXTXXEKPAAKLYPQAZESREVSDR 97  
 101 SRRRGPCPCLPEWDNIVCWPLGAPGEVVAVPCPDYIYDPNHKGAYRRCDR 150  
 98 SRLQDGFCLPEDNIVCWPGKVVAVPCPDYFYDPNHKGAYRRCDS 147  
 151 NGSWEVVPGHNRTHANYSECLKFMTNETREREVFDRLGMIYTGYSMSLA 200  
 148 NGSWELVPGNNRTHANYSECVKPLNETREREVFDRLGMIYTGYSISLG 197  
 201 SLTVAVLILAYFRRRHCTRNYIHMHMPLSFMLRAASIFVKDAVLYSGFTL 250  
 198 SLTVAVLILCYFRRRHCTRNYIHMHLFVFSFMLRAVSIFIKDAVLYSGVST 247  
 251 DEAEERLTSEEELHIIAQVPPPPAAAAAVGYAGCRVAVTFFFLYFLATNYYWIL 300  
 248 DEIERITEELRAFTE...PPPDKAGFVGCRVAVTVFLYFLTNYYWIL 294  
 301 VEGLYLHSILFMAFFSEKKYLWGFTI FGHGLFAVFVAVWVGV RATLANTC 350  
 295 VEGLYLHSILFMAFFSEKKYLWGFTLFGHGLFAVFVAVWVTV RATLANTE 344  
 351 CWDLSSGKRWIIQVPILASVVLNEILFINIIRVLATKLRETNAGRCCTR 400  
 345 CWDLSSGNRKWI IQVPIAAIVVN FILFINIIRVLATKLRETNAGRCCTR 394  
 401 QOYRKLLRSTLVLPPLFGVHYTVFMALPYTEVSGTLWQIQMHYEMLFNSF 450  
 395 QOYRKLLKSTLVLPPLFGVHYIVFMATPYTEVSGILWQVQMHYEMLFNSF 444  
 451 CGFFVAIIZYCTCNGEVQAEIRXWSRWTI ALDFRKARSGSSSYSGPMV 500  
 445 CGFFVAIIZYCTCNGEVQAEIXXWSRWTI ALDFRKARSGSSSYSGPMV 494  
 501 SHTSVTIVGFRAGLSSLPLSPRLPP...ATTNGHSOLPGHAKPGAPATEZ 547  
 495 SHTSVTIVGFRGGLALSLSPRLAPGAGASANGERQLPGYVKEGSISENSL 544  
 548 TL2VTMAVPKDDGFLNGSCSGLDEEASGEAAPPPLLQEGWETVM. 591  
 545 PSSGZEPGTXDDGYLNQ..SGLYEPMVG.EQ777PLLEERETVM. 586

Gap Weight: 3.000      Average Match: 0.540  
 Length Weight: 0.100      Average Mismatch: -0.396

Quality: 712.2      Length: 595  
 Ratio: 1.215      Gaps: 5  
 Percent Similarity: 87.113      Percent Identity: 77.835

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Fig. 5

R15	MGAARIAPSL	ALLLCCPVLS	SAYALVDADD	VITKEEIQIFL	LERAQAQCDK	50
Oko	MGAPRISHSL	ALLLCCSVLS	SVYALVDADD	VITKEEIQIL	LRNAQAQCEQ	50
Okh	MGAPRISHSL	ALLLCCSVLS	SVYALVDADD	VITKEEIQIL	LRNAQAQCEQ	50
----- A -----						
R15	LLKEVLHTAA	NIMESDRKGWT	PASTSGKPRK	EXASGKFYPE	SKENKDVTPTG	100
Oko	RLKEVLR.VP	E LAESAKDW.	.MSRSAKTKK	EXPAAEKLYPO	AEEESREVSDR	97
Okh	RLKEVLR.VP	E LAESAKDW.	.MSRSAKTKK	EXPAAEKLYPO	AEEESREVSDR	97
*						
R15	SRRRGRCPLP	EWDNIVCWPL	GAPGEVVAVP	CPDYIYDFNE	KGHAYRRCDR	150
Oko	SRLQDGFCLP	EWDNIVCWPA	GVPGKVVAVP	CPDYFYDFNH	KGRAYRRCDS	147
Okh	SRLQDGFCLP	EWDNIVCWPA	GVPGKVVAVP	CPDYFYDFNH	KGRAYRRCDS	147
----- B -----						
N N N N						
R15	NGSWEVVPGH	NRTWANYSEC	LKFMTNETRE	REVFDRLGMI	YTGVYSMSLA	200
Oko	NGSWELVPGN	NRTWANYSEC	VKFLTNETRE	REVFDRLGMI	YTGVYSISLG	197
Okh	NGSWELVPGN	NRTWANYSEC	VKFLTNETRE	REVFDRLGMI	YTGVYSISLG	197
-----						
R15	SLTVAVLILA	YFRRRHCTR	YIHMRRMFLSF	MLRAASIFVK	DAVLYSGFTL	250
Oko	SLTVAVLILG	YFRRRHCTR	YIEMELFVST	MLRAVSIFIK	DAVLYSGVST	247
Okh	SLTVAVLILG	YFRRRHCTR	YIEMHLFVST	MLRAVSIFIK	DAVLYSGVST	247
-- C ----- D -----						
R15	DEAERLTSEE	LHIIAQVPPP	PAAAAVGYAG	CRVAVTFPLY	FLATNYYWIL	300
Oko	DEIERITEEE	LRAFTE...P	PPADKAGFVG	CRVAVTVFLY	FLTTNYYWIL	294
Okh	DEIERITEEE	LRAFTE...P	PPADKAGFVG	CRVAVTVFLY	FLTTNYYWIL	294
----- E -----						
R15	VEGLYLHSLI	FMAFFSEKKY	LWGFTIFGWG	LPAVFTVAVWV	GVRATLANTG	350
Oko	VEGLYLHSLI	FMAFFSEKKY	LWGFTLFGWG	LPAVFTVAVWV	TVRATLANTE	344
Okh	VEGLYLHSLI	FMAFFSEKKY	LWGFTLFGWG	LPAVFTVAVWV	TVRATLANTE	344
---- F ----- G -----						
R15	CWDLSSGHKK	WIIQVPILAS	VVNLNPFILFIN	IIRVLATKLR	ETNAGRCDT	400
Oko	CWDLSSGNKK	WIIQVPILAA	IVVNPFILFIN	IIRVLATKLR	ETNAGRCDT	394
Okh	CWDLSSGNKK	WIIQVPILAA	IVVNPFILFIN	IIRVLATKLR	ETNAGRCDT	394
----- H -----						
R15	QQYRKLLRST	LVLVPLFGVH	YTVMALPYT	EVSGTLWQIQ	MHYEMLFNSF	450
Oko	QQYRKLLRST	LVLMPPLFGVH	YIVFMATPYT	EVSGILWQVQ	MHYEMLFNSF	444
Okh	QQYRKLLRST	LVLMPPLFGVH	YIVFMATPYT	EVSGILWQVQ	MHYEMLFNSF	444
----- I -----						
R15	QGFFVIAIYC	FCNGEVOAEI	RKSWSRWTLA	LDFKRKARSG	SSSYSYGPMV	500
Oko	QGFFVIAIYC	FCNGEVOAEI	KKSWSRWTLA	LDFKRKARSG	SSTYSYGPMV	494
Okh	QGFFVIAIYC	FCNGEVOAEI	KKSWSRWTLA	LDFKRKARSG	SSTYSYGPMV	494
-- J -----						
R15	SHTSVTNVGP	RAGLSPPLSP	RLPP...ATT	NGHSQLPGEA	KPGAPATE	547
Oko	SHTSVTNVGP	RGGLALSLSP	RLAPGAGASA	NGHBQLPGYV	RHGSISENSL	544
Okh	SHTSVTNVGP	RGG.....	....WPCPSA	LD		515
-----						
R15	TLPVTMAVPK	DDGFLNGSCS	GLDEEASGSA	RPPPLLQEGW	ETVM	591
Oko	PSSGPEPGTK	DDGYLNG..S	GLYEPMVG.E	QPPPLLEER	ETVM	585

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FIG. 5

With 1 enzymes: SACI

February 27, 1992 18:30 ..

GGGATCCCGCGGCCCTAGGCGGTGGCGatççgçGAccGCccçgatcçcacccggectggcg  
 2 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 CCCTAGGGCGCCGGGATCCGCCACCGCtácccCTggCGggcczazççtggccggaccgc  
 M G T A R I A P G L A -  
 ctcccçgctctgctçcccgtgctcagctccçççtacççççtggatçgagatçacgtc  
 62 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 gagggacgagacgacggggcacçgagtcçagggççatgçgcçaccacctacgtctactgcag  
 L L L C C P V L S S A Y A L V D A D D V -  
 atçactaaaqçgaaacagatcttccççtçccççtcaaggcccagtgcgaaaaacgg  
 122 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 çactçatttctcczçtctagaaggacççtçççagtcçgggtæacgcttttggcc  
 M T K E Z Q I F L I H R A Q A Q C E K R -  
 ctcaaçgaggccççcagaggccagccagcatatççaaatcaqacaagggtatggacatct  
 182 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 gagttccctccaaaggacççtccççgtcççtattsccttagtçtçctacctçttaga  
 L K E V I Q R P A S I M E S D K G W T S -  
 gçgtccacatcaaggaaagcccaggaaaqataaççcatctgggaaggtctaccctgagtct  
 242 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 çççagggtgttagtcccccgggtcccccattccçtagacccttsgagatgggactcaga  
 A S T S G K P R K D Y A S G K L Y P E S -  
 caaggaggacaqaççagççaccactççcaççatccçagçççcccctgtetçccggaa  
 302 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 ccctccctçtccctccctççgtçaccçtcccccçtcccccçtcccccçggggacagacggccct  
 E E D K S A P T G S - . A S R P C L P S -  
 tçççaccacatccççtççtççccçtççggccçtççggccçtcccccçtcaqgtçgtçgtçtgc  
 362 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 accctççgtçtaggacacgaccçççgçaccçccçtççtccactccaccacçgacacgggaca  
 W D H I L C W F L G A F S E V V A V P C -  
 ccçgactacatttatçacttcaatcacaaaqçccatçccctaccçgacgctgtgaccgcaat  
 422 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 ççccççqatçtaaaatactçgaaqtttagtçtccççtacççgatggctçgçgacactggcgtta  
 P D Y I Y D F N H K G A A Y R R C D R N -  
 çççcaçctççgaaçtççtççtççggcacaacaççacçtçggccaaactacagçgagtggt  
 482 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 çççtççaccçtççaccacggaccççtçtçtcccccçtcccccçtççtçtçtçtçtçtçtçtç  
 541

S 3 C -

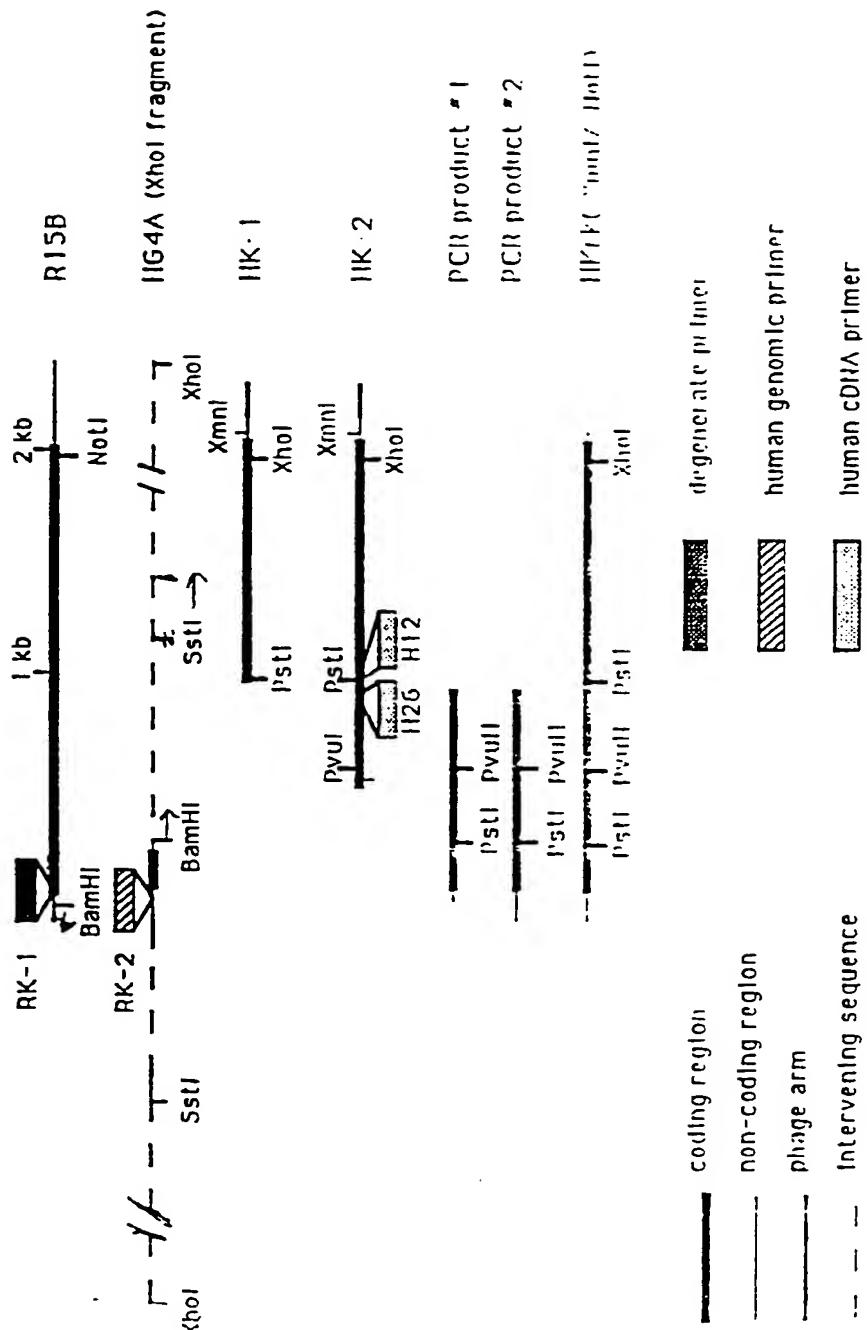


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### Enzymes that do cut:

Saci

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Fig.3

PERIOD of: 100 dep clk: 7509, 1 to 594 March 7, 1992 16:26  
1000 AVE of: 100 dep clk: 9754 from: 29 to: 1010

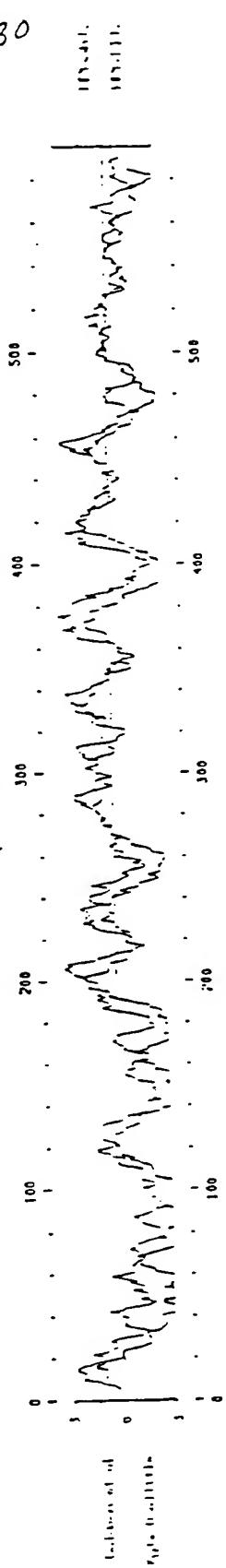
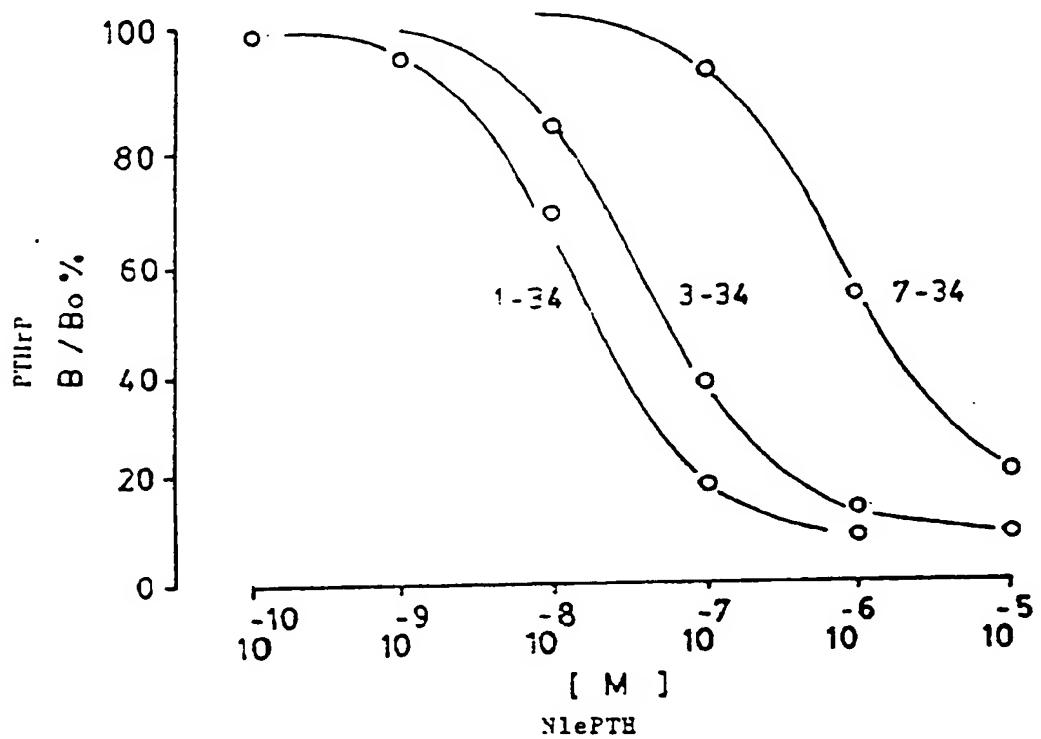
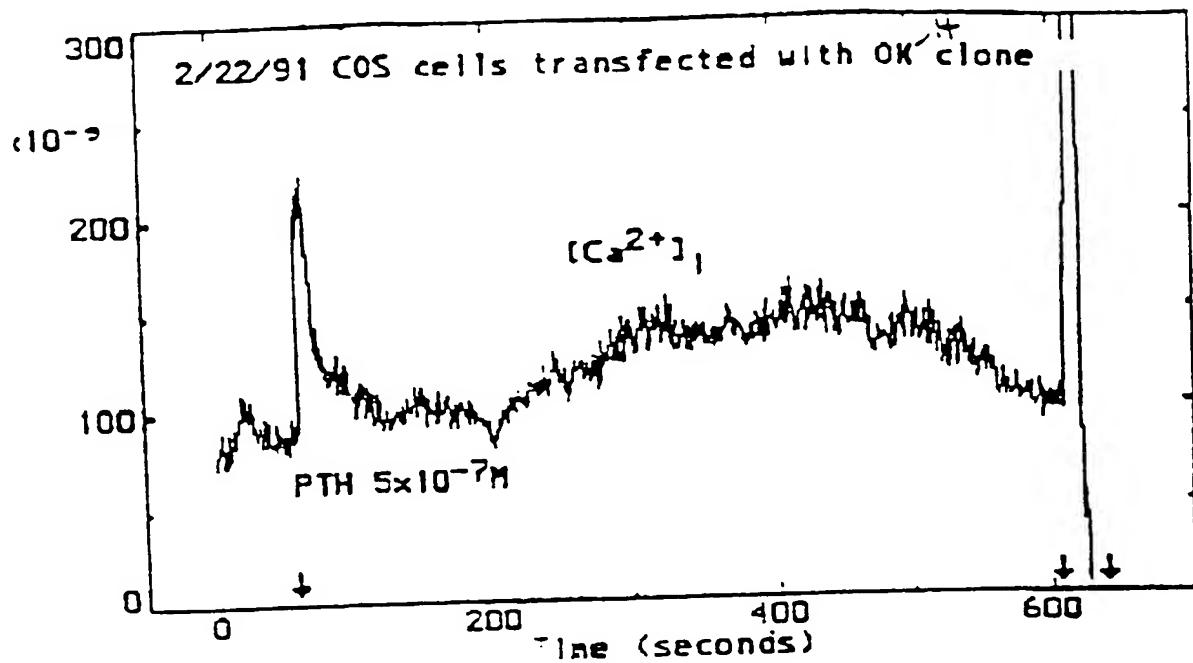


FIG. 9



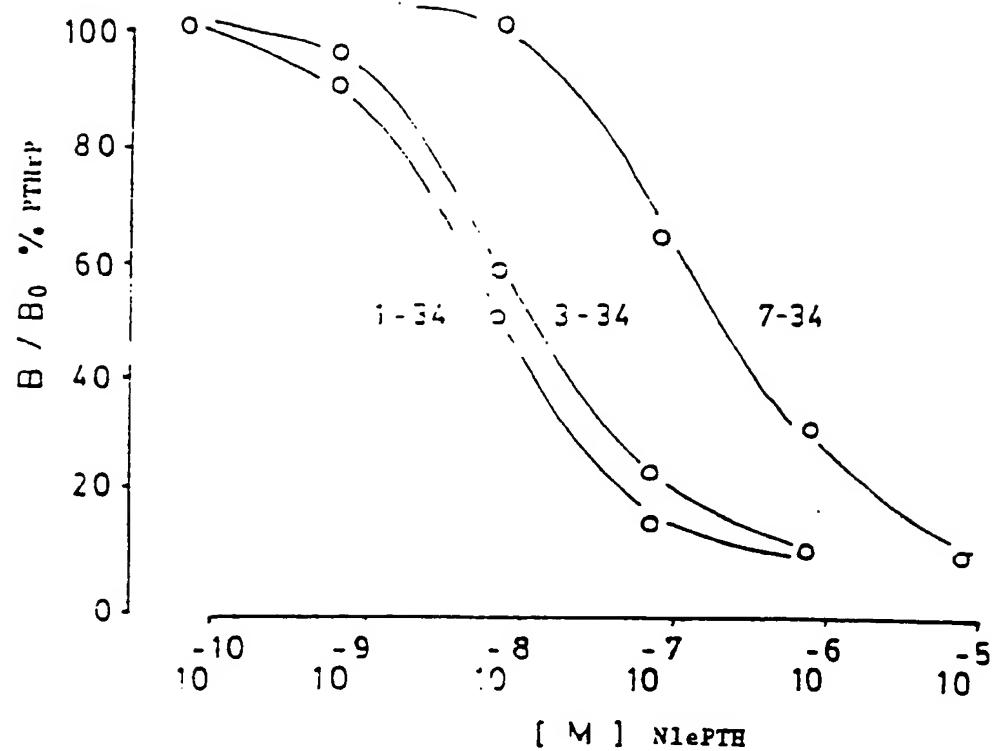
19/30

FIG. 10



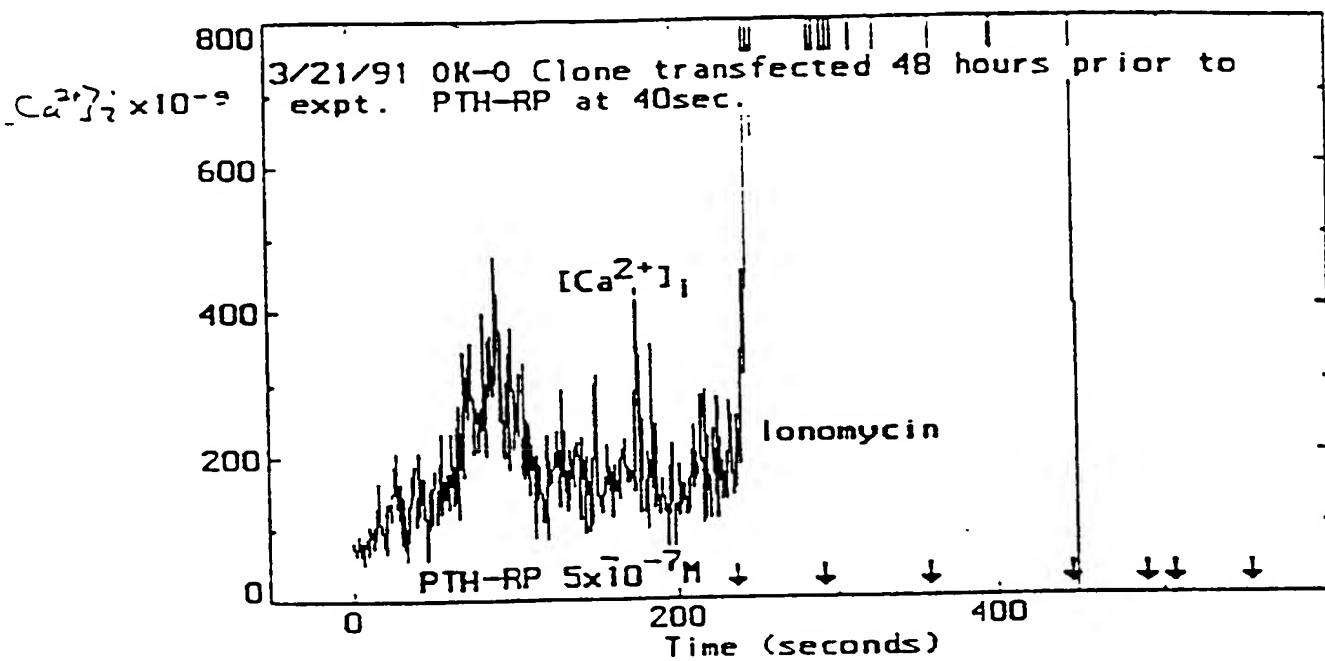
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Fig. 11



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FIG.12



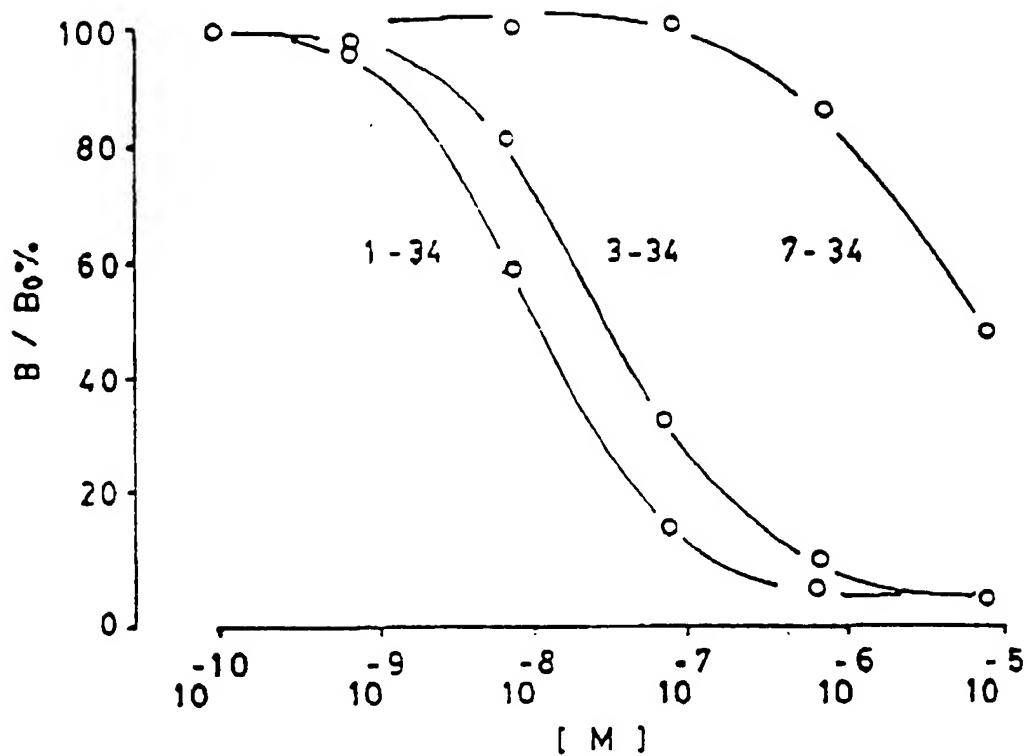
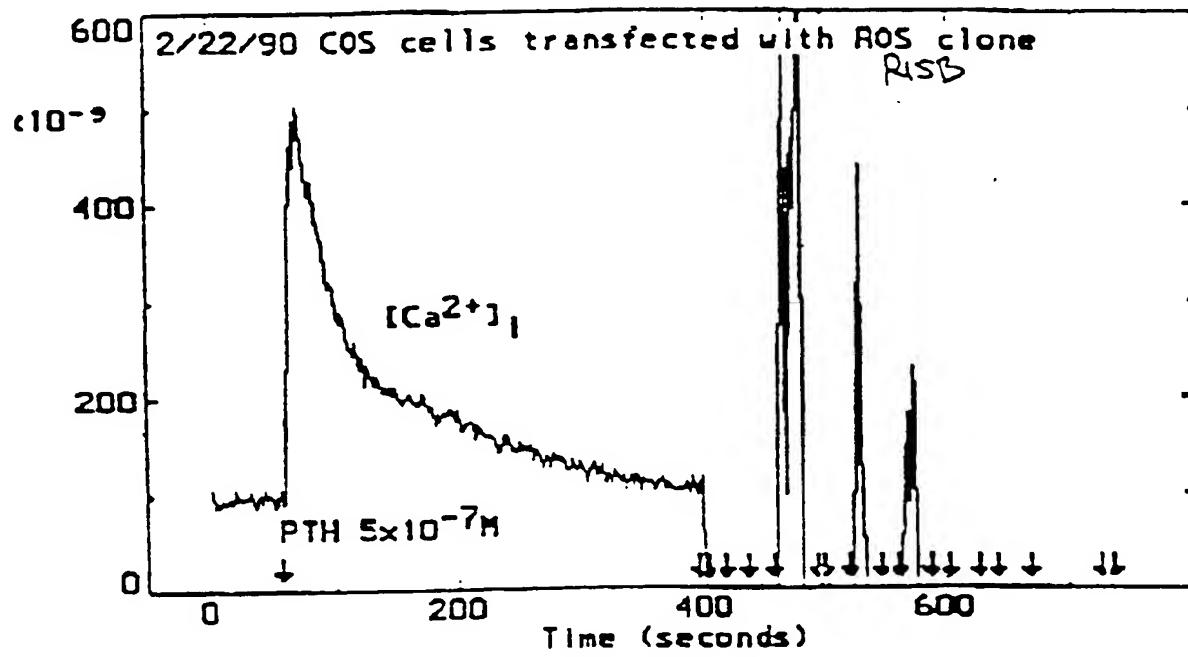


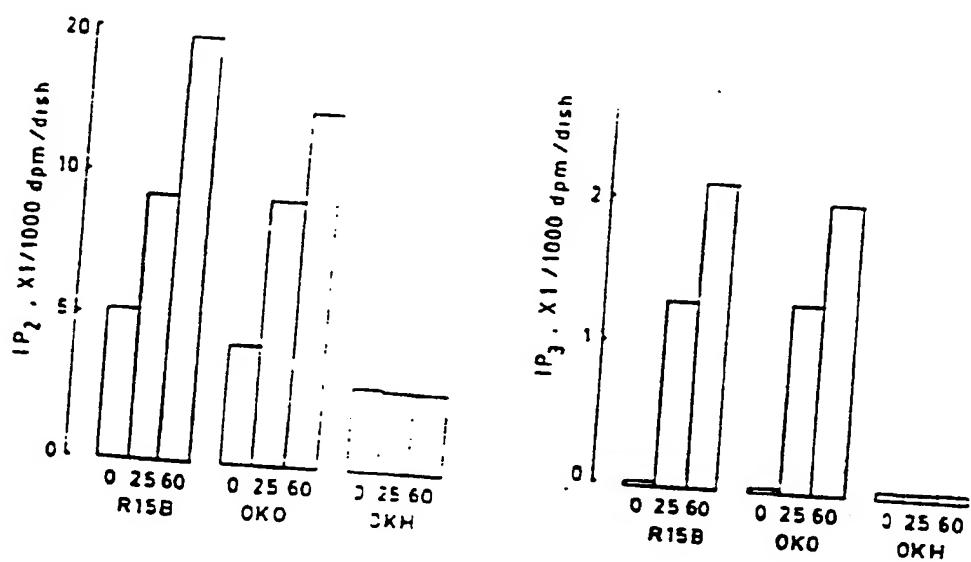
FIG. 13

FIG. 14



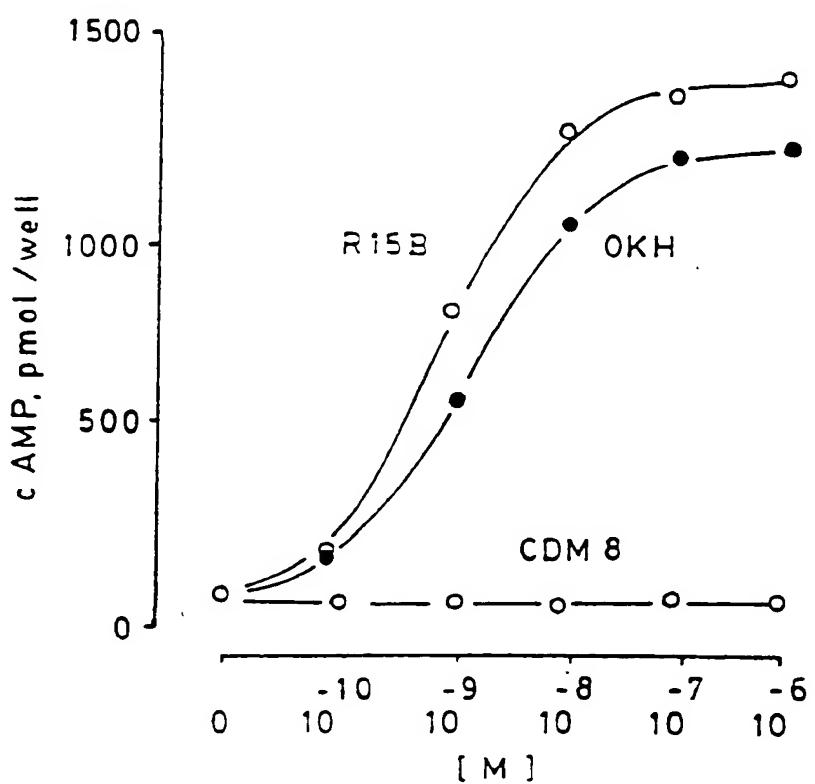
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FIG. 15



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FIG. 16



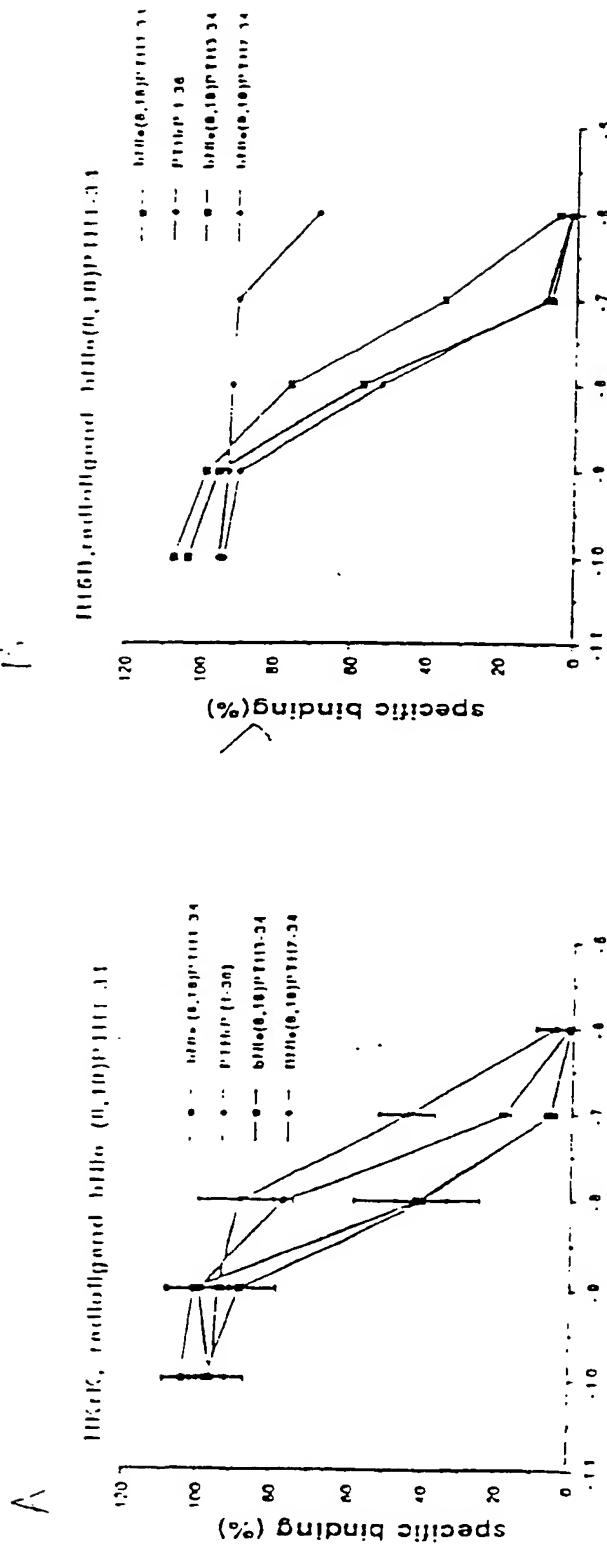
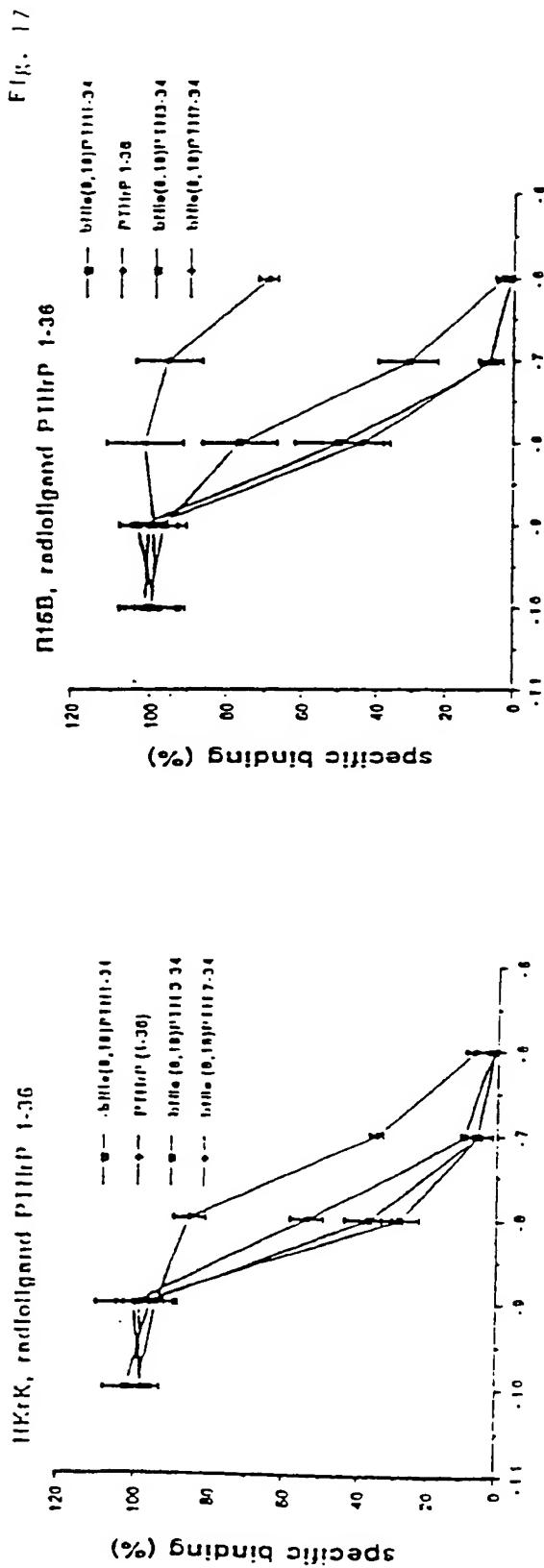
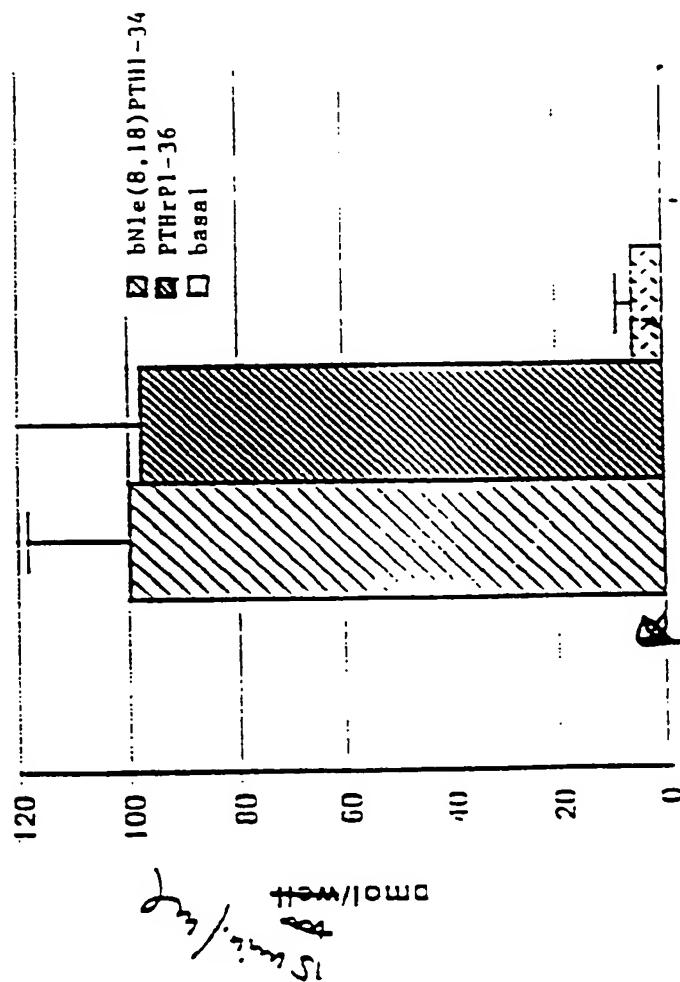


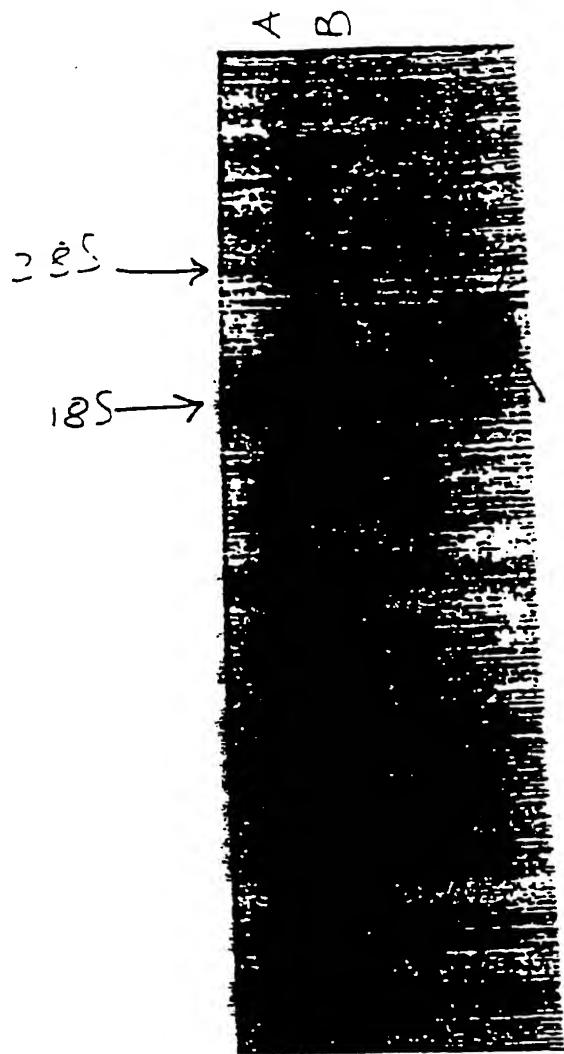
Fig. 18

213°



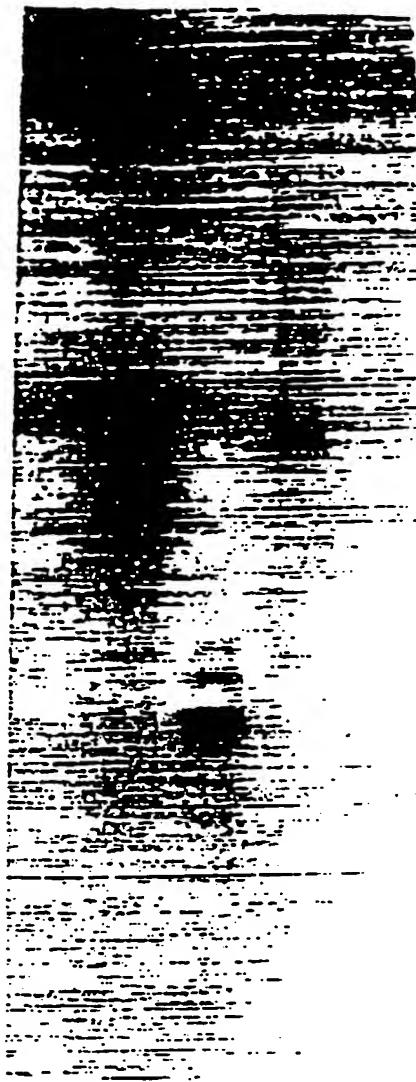
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Fig. 19



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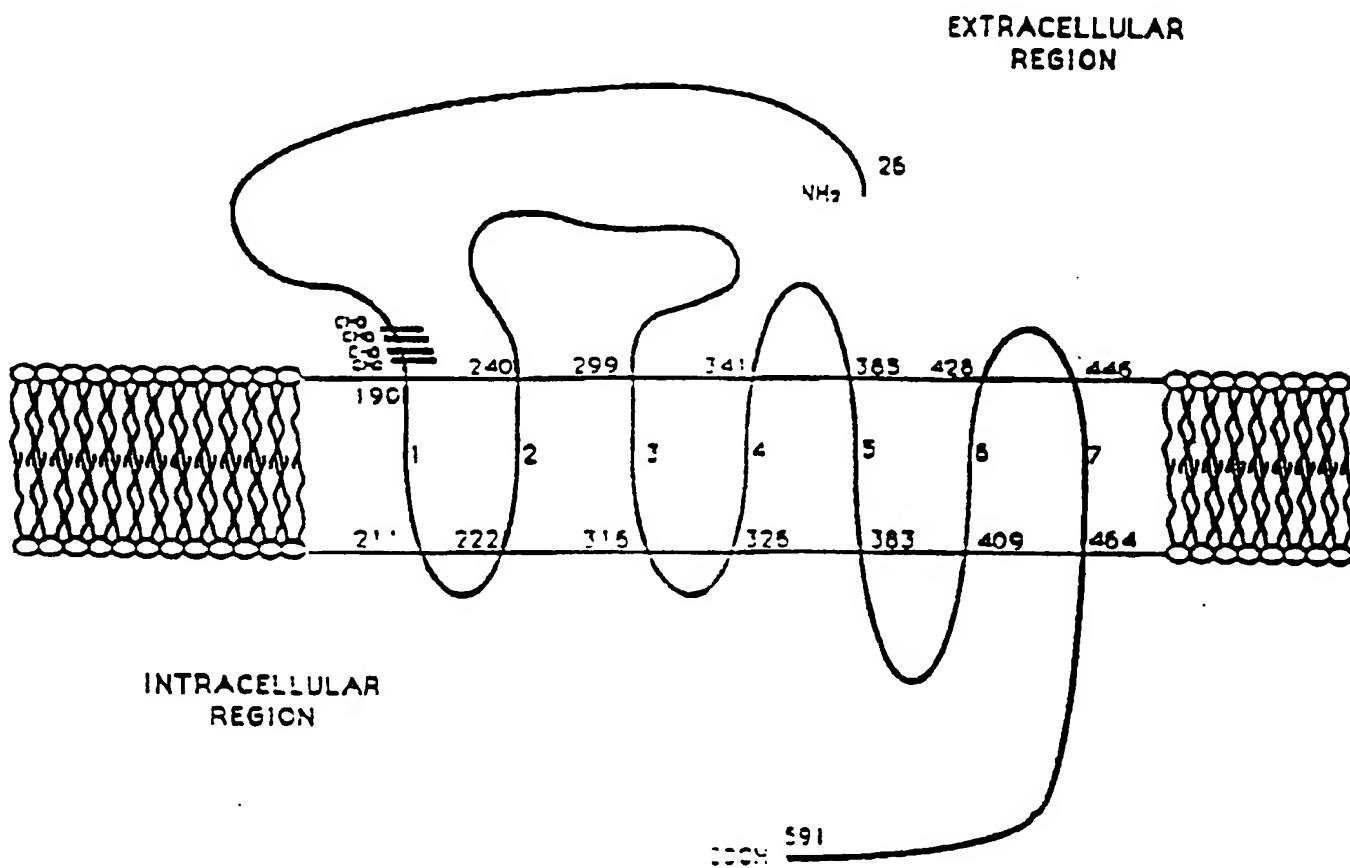
Fig. 20



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Fig. 21

## RAT BONE PTH/PTHrP RECEPTOR



## AMINO ACID SEQUENCE OF 7 PUTATIVE TRANS-MEMBRANE REGIONS

1	2	3
VGYSMSLAS-TVAVLILAY	-IHMHMFLSFMLRAAS-FVK-	-LVEGLYLMHSIFMAFFS-
6	5	4
STLVLVPLFSGVHVTVFMALP	-VP:LASVVVNLIFINIR-	-IFGWGLPAVFVAVWVGV
7		
MLFNSFOGFFVAVIYCFN		

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/02821

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :Please See Extra Sheet.

US CL :435/69.1, 240.2, 320.1; 536/27, 28, 29; 530/350, 387, 397, 399.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : APS AND COMMERCIAL DATABASES (DIALOG) 435/69.1, 240.2, 320.1; 536/27, 28, 29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG AND ONLINE SEQUENCE SEARCH

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	TWENTY-SEVENTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, VOLUME 105, NO. 4, PT. 2, ISSUED OCTOBER 1987, R. A. LUBEN ET AL., "MOLECULAR CLONING OF A PARATHYROID HORMONE RECEPTOR-RELATED MEMBRANE PROTEIN FROM MOUSE BONE CELLS", ENTIRE DOCUMENT.	1-19, 39 20-38, 40-49
Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL 265, NO. 1, ISSUED 05 JANUARY 1990, ABOU-SAMRA ET AL., "CHARACTERIZATION OF FULLY ACTIVE BIOTINYLATED PARATHYROID HORMONE ANALOGS", PAGES 58-62, ENTIRE DOCUMENT.	1-49 -
Y	BIOCHEMISTRY, VOLUME 29, NO. 30, ISSUED 31 JULY 1990, JUPPNER ET AL., "PREPARATION AND CHARACTERIZATION (N-(4-AZIDO-2-NITROPHENYL)ALA. TYR-36)-PATHYROID HORMONE RELATED PEPTIDE (1-36) AMIDE: A HIGH-AFFINITY, PARTIAL AGONIST HAVING HIGH CROSS-LINKING EFFICIENCY WITH ITS RECEPTOR ON ROS 17/2.8 CELLS", PAGES 6941-6946, ENTIRE DOCUMENT.	1-49

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 JULY 1992

Date of mailing of the international search report

31 JUL 1992

Name and mailing address of the ISA/  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No.

Authorized officer

GIAN WANG

Telephone No. (703) 308-3993

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/02821

A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (5):

C12P 21/06; C12N 5/00, 15/00; C07H 15/12, 17/00; C07K 3/00; A61K 35/14, 37/24, 37/36.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING